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Pulmonary and Thoracic Macrophage Subpopulations and Clearance of Particles from the Lung

by Bruce E. Lehnert[1]

Pulmonary macrophages consist of several subpopulations that can be defined by their anatomical locations as well as by other criteria. In addition to the well-known alveolar macrophages that reside on the alveolar surface, pulmonary macrophages also occur in the conducting airways, in various pulmonary interstitial regions, and, in some mammalian species, in the lung's intravascular compartment. Other thoracic macrophages of relevance to pulmonary defense and some lung disease processes are the pleural macrophages resident in the pleural space and macrophages present in regional lymph nodes that receive lymphatic drainage from the lung. Of the above subpopulations of pulmonary and thoracic macrophages, the alveolar macrophages have received the most experimental attention in the context of the pulmonary clearance and retention of deposited particles. Accordingly, less information is currently available regarding the roles other pulmonary and thoracic populations of macrophages may play in the removal of particles from the lower respiratory tract and associated tissue compartments. This report provides an overview of the various subpopulations of pulmonary and thoracic macrophages, as defined by their anatomical locations. The known and postulated roles of macrophages in the pulmonary clearance and retention of particles are reviewed, with particular emphasis on macrophage-associated processes involved in the pulmonary clearance of relatively insoluble particles.

Introduction

Macrophages in the lower respiratory tract and associated tissues consist of several subpopulations that can be defined by their anatomical locations as well as by other criteria. Based on location only, the pulmonary or lung macrophages can be classified as alveolar macrophages that reside on the epithelial surfaces of the alveoli, airway macrophages that are found on and in the epithelial lining of the conducting airways and in bronchus-associated lymphoid tissue, and interstitial macrophages that are located in perivascular, peribronchiolar, and visceral pleural sites, as well as in the interstitium of the alveolar region. In addition to these pulmonary macrophage subpopulations, bona fide macrophages also exist in the pulmonary vascular compartment of some mammalian species. Other thoracic populations of macrophages that are relevant to pulmonary defense and some disease processes include the pleural macrophages that reside in the pleural space and macrophages that are present in regional lymph nodes that receive lymphatic fluid drainage from the lung.

The objective of this report is to provide a brief overview of the above populations of macrophages in the context of the roles they play in both the removal and retention of particles that deposit in the lower respiratory tract, as well as to point out some existing gaps in the current understanding of their functional roles following particle deposition in the lung. Particular emphasis is placed on macrophage-associated particle clearance and retention processes involving relatively insoluble particles.

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Pulmonary Macrophages

Alveolar Macrophages

Overview. The alveolar macrophages (AM) reside on the alveolar epithelial surface (*1*), where they provide phagocytic defense against deposited particles and perform a variety of other functional activities (*2–7*). Numerically, the AM account for the majority of the pulmonary macrophages present in the lungs of mammalian species that do not contain abundant, intravascular macrophages (to be discussed). Under normal conditions, the AM represent about 3–5% of all the cells in the alveolar region of the lungs of rats, dogs, baboons, and nonsmoking humans (*8–10*). In the rat, the AM account for about 60% of the total number of all macrophages in the lung (*10*). Of all the subpopulations of macrophages in the lung, the AM have been most extensively studied mainly because they can be simply obtained by bronchoalveolar lavage in high states of purity.

The lung's AM can be defined as being a relatively homogeneous and distinct subpopulation of macrophages because of their common location on the alveolar surface and because they have been shown to possess unique antigens not found with other body or pulmonary macrophage subpopulations (*11–15*). Even so, many studies have demonstrated marked heterogeneity among AM harvested by bronchoalveolar lavage in terms of their morphologic, biophysical, biochemical, and functional characteristics, as well as in terms of their cell surface profiles of antigens and other constituents (*13,16–24*). The basis of this diversity has not been clearly resolved, but such AM heterogeneity

may be due to the coexistence of cells in various stages of differentiation or maturation that are derived from a common mononuclear cell lineage and/or due to the presence of subset populations of AM derived from macrophage precursors of distinctly separate cell lineages (*25,26*). Several technically diverse approaches have been used to examine AM heterogeneity (*17,21,22*). Because AM range widely in size, one general approach commonly used to fractionate lavaged AM populations into subset categories defined by their size and density is density gradient centrifugation. *In vitro* analyses of density-defined subpopulations of AM, in conjunction with other lines of evidence (*27*), including the size changes blood monocytes undergo as they differentiate into macrophages *in vitro* (*28–30*), have led numerous investigators to postulate that the size of AM correlates with cell age, with the largest or least dense AM being the most mature (*26,31*). This conclusion implies that the heterogeneity in AM size, at least, is due to the overall AM population being composed of macrophages at different stages of maturation, but it ignores the possibility that differences in the sizes and other features of AM may as well be related to their precursor source(s), which are numerous. Migration of peripheral blood monocytes into the alveoli, migration of interstitial macrophages into the alveoli, and the local proliferation of resident AM have all been postulated as being major processes by which stability in the AM population size is maintained as AM are continuously removed from the lung under steady-state conditions (*32–46*). Additionally, environmentally related factors in the alveoli also may substantially contribute to the physical and functional characteristics and heterogeneity of the lung's AM (*47–49*). Whether different subpopulations of AM, however defined, have different roles in the clearance and retention of particles that deposit in the alveoli remains to be determined. Some *in vitro* studies in which the functions of density-defined AM subpopulations have been examined, however, suggest that the least dense and most dense AM are somewhat less capable of phagocytizing some types of particles (*16,31*), and they migrate less actively in response to chemotactic stimuli compared to their more mid-range density counterparts (*16,50,51*).

AM Responses to Particle Deposition. One well-recognized defensive function of AM is the phagocytosis or engulfment of particles that deposit in the alveolar region. Clearly, the AM are capable of phagocytizing a wide variety of materials within hours after deposition (*6,52*). Most of the particles in the alveoli are engulfed within a 24-hr period after a single bout of particle deposition (*6,53*) as long as the burden of deposited particles does not overwhelm the capacity of alveolar phagocytes to ingest them. The phagocytic function initially serves to sequester particles from the alveolar lining fluid and alveolar epithelial cells. Obviously, the process of particle phagocytosis requires particle–AM encounters as a first step, with such encounters being a major factor limiting the rate and efficiency by which particles may be engulfed by the AM (*54*). While particle–AM encounters may occur due to the random mobility of AM on the alveolar surface, theoretical considerations of the rapidity by which AM engulf particles *in vivo* suggest that a directed migration of AM to the particles results from locally generated chemotactic factors (*55*). This possibility has gained support from the work of Warheit and co-workers (*56–58*), who demonstrated that particulate asbestos, which activates complement to form the chemoattractant C5a, brings about the recruitment of macrophages to the sites where the asbestos initially deposits in the lung. On the other hand, the rapidity with which AM phagocytize and hence encounter particles in the alveoli may to some extent be independent of macrophage movement. The results of a recent investigation by Schurch and co-workers (*59*) suggest that physical force interactions between deposited particles and the alveolar lining fluid causes particles to enter the subphase of the lining fluid and that gradients in subphase pressure and/or capillary action may move the subphase material to alveolar corners where AM preferentially reside (*60*). The relative importance the pores of Kohn may play in the phagocytosis of deposited particles by providing a pathway for the migratory passage of AM from one alveolus to another (*61*) remains unclear.

In addition to engulfing particles that land on the alveolar surface, AM can also acquire particles by phagocytizing other particle-containing phagocytes (*62*). This process may be especially important as a mechanism for removing particle-laden polymorphonuclear leukocytes (PMN) that were recruited in response to the deposition of particles. As shown in Figure 1, most of an intratracheally instilled burden ($\sim$3.7 mg) of polystyrene microspheres is contained predominantly in alveolar PMN after 6 hr. During the subsidence of the PMN response, particle-containing PMN are seen in the AM (Fig. 2), and when the PMN response has essentially totally subsided and only a small fraction of the lung burden has cleared from the lung, virtually all of the particles retained in the alveoli are contained in AM (*63,64*). Similarly, particle burdens in the AM can also be increased when they phagocytize other AM (see Fig. 8).

While serving the function of particle containment, another important outcome of the phagocytosis of some types of particulate materials is the stimulation of the AM to elaborate chemotactic factors, e.g., complement components, tumor necrosis factor, leukotriene B_4, and other arachidonic acid metabolites (*21,65–73*), and perhaps mitogenic factor(s) (*74*), that can lead to increases in the numbers of phagocytes available in the alveoli, i.e., "the free-cell response." Some lines of evidence, moreover, suggest that free radicals released by phagocytizing AM that are undergoing the respiratory burst can also result in the formation of potent chemotactic factors upon reaction with various biochemical constituents in the alveolar extracellular lining fluid (*75,76*).

In response to the AM-derived chemotactic factors and/or chemotactic stimuli that may be generated by direct interactions of particles with constituents in the lung's lining fluid (*57,58,74*), the initial phase of the free-cell response is typically characterized by the recruitment of PMN from the pulmonary vasculature into the alveolar space compartment (*35,62,77,78*). Shortly after this early response, which begins within hours after particles are deposited, AM numbers increase as blood monocytes migrate from the pulmonary vasculature and, probably to a lesser extent, as the result of an influx of macrophages from interstitial sites in the alveolar region. Unlike the PMN response, which is relatively immediate and lasts for only a few days, the expansion of the AM population size is longer lived (*35,62*), with the early increases in AM numbers being sustained mainly by the subsequent proliferation and migration of interstitial macrophages into the alveoli (*35,77*). The extent to which the intra-alveolar replication of AM (*39,41,42,79*) resident in the alveoli at the time of parti-

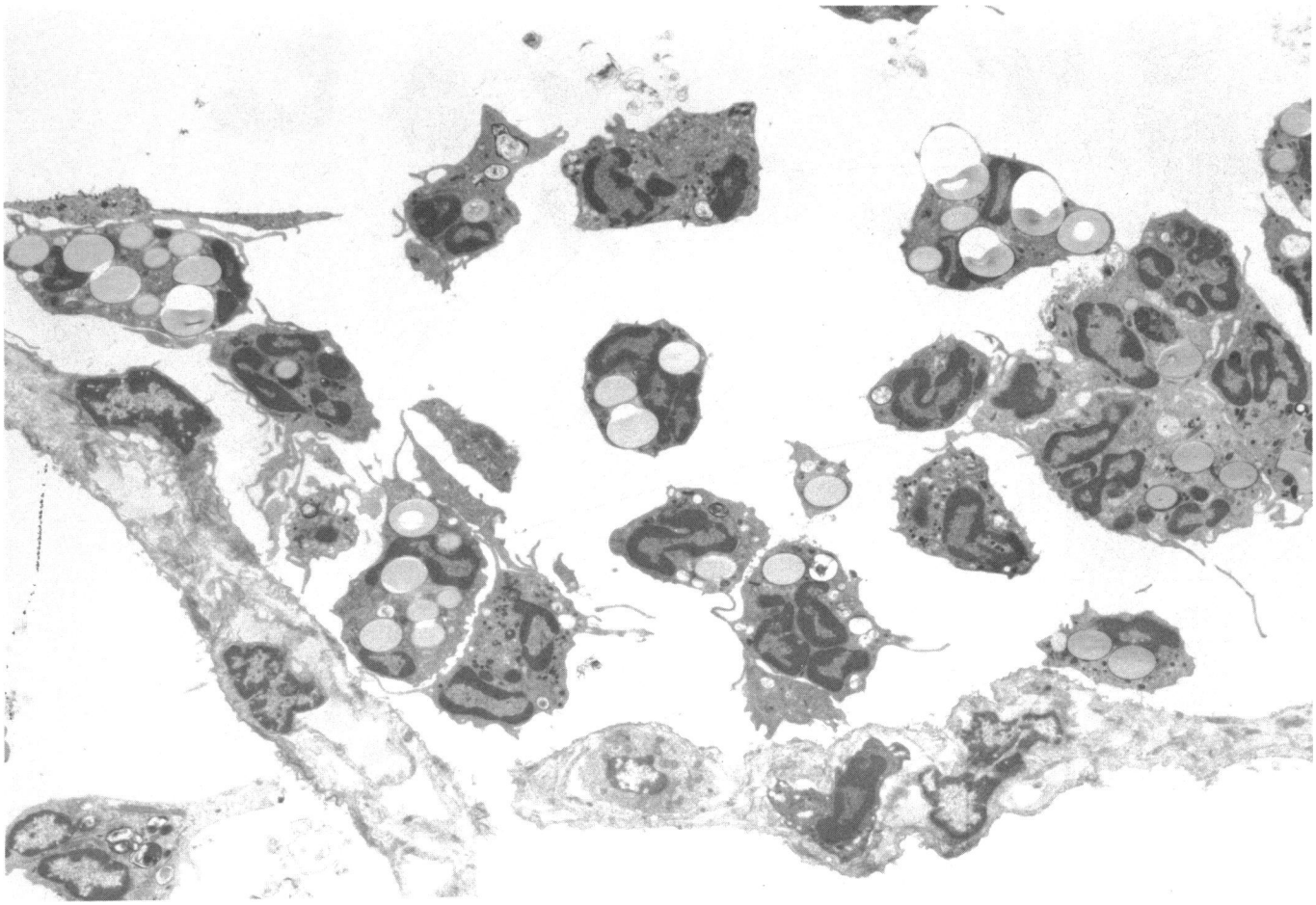

FIGURE 1. Transmission electron micrograph of the alveolar region of a rat's lung 6 hr after the intratracheal instillation of polystyrene microspheres (3.7 mg). Most of the particles have been phagocytized by recruited polymorphonucleated leukocytes.

cle deposition and/or the replication of newly recruited macrophages may contribute to the elevation of AM numbers during the free-cell response requires further study. Regardless, both the particulate load of a material deposited in the lung as well as the sizes of the particles deposited are important factors that affect the magnitude of the free-cell response. Generally, more phagocytes are recruited into the alveoli as acutely deposited lung burdens increase (*80*), and smaller and larger particles delivered to the lung in equivalent masses result in greater and lesser elevations in the free-cell population size, respectively (*80,81*). Overall, the role AM play in releasing chemotactic factors for the recruitment of more phagocytes can be most simply viewed as a response that has evolved to provide more professional phagocytes in the alveoli to ensure that deposited particles do not escape the phagocytic mechanism. Indeed, with the exception of extraordinary experimental conditions in which the magnitude of a deposited lung burden of particles exceeds the lung's ability to mount a free-cell response with enough phagocytes to engulf the vast majority of particles (*81*), and aside from conditions in which the cytotoxicity of deposited materials result in an actual decrease in available phagocytes (e.g., *82–88*), the recruitment of PMN and macrophages during a typical free-cell response to particle deposition is exaggerated so that more phagocytic cells are made available than are required to sequester the particles (*62*).

Roles of Particle Clearance and Retention. The predominant mechanism by which insoluble particles are thought to be removed or cleared from the lung is by the cephalad transport of AM with their phagocytized burdens of particles up the conducting airways. As end points of this translocation pathway, the AM and particles are either swallowed or expectorated. Recognition of this "AM-associated particle clearance" or "AM-mediated particle clearance" process is mainly based on circumstantial evidence, such as observations of particle-containing macrophages on the luminal surfaces of airways at times well after the particles were deposited in the lungs (*89–91*), and on inferential extensions of experimental results that have shown that the bulk of particles retained in the lung are associated with AM over the course of alveolar phase clearance (*6,63,91*). Further support for AM-associated particle clearance via the airways comes from findings that insoluble particles removed from the lung during alveolar phase clearance can be quantitiatively accounted for in the gastrointestinal tract and feces (*92,93*).

How the AM gain access and become coupled to the ciliated airways for cephalad transport on the mucociliary apparatus (Fig. 3) has not been conclusively determined, although numerous

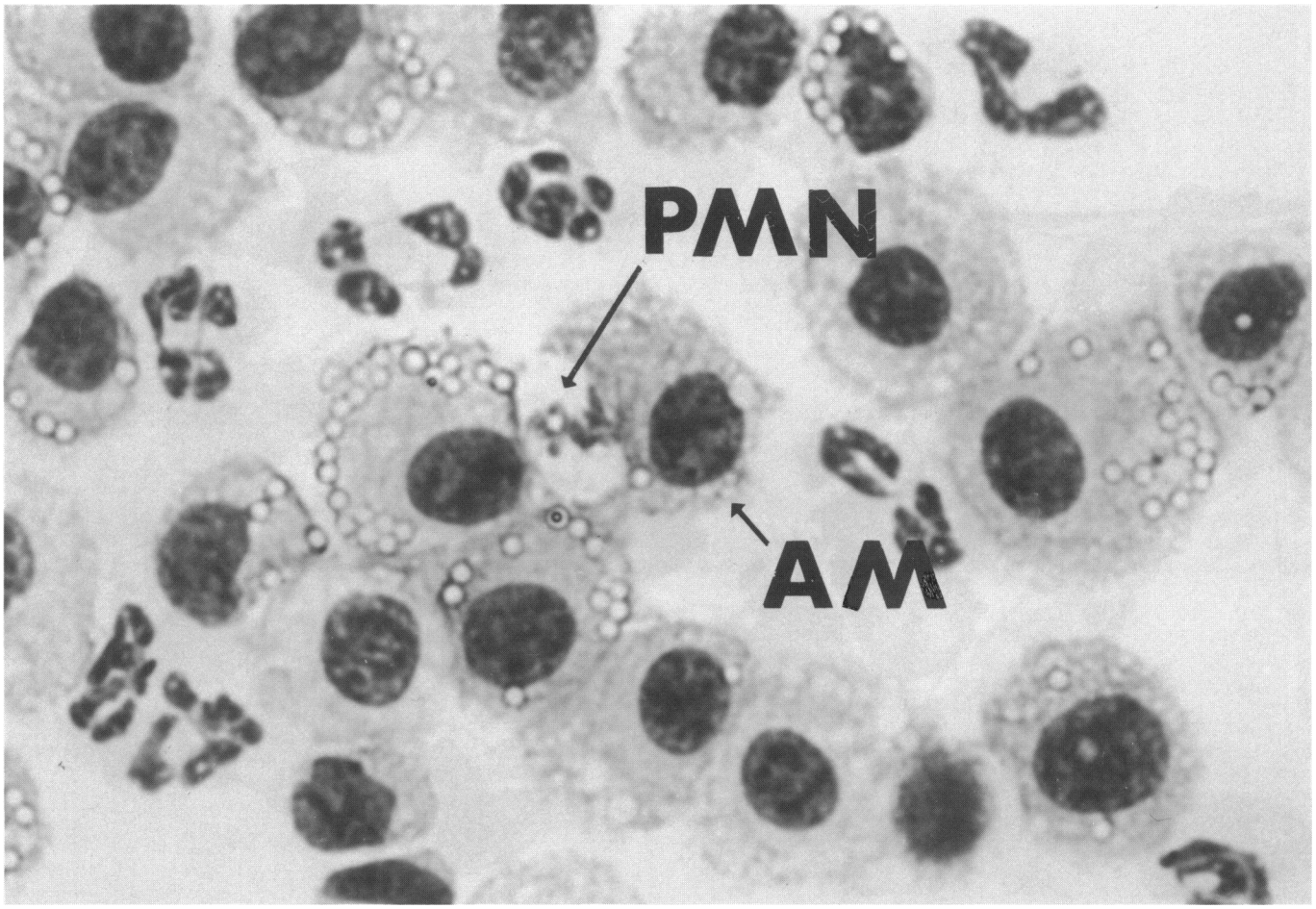

FIGURE 2. Micrograph of lung free-cells lavaged from a rat's lung 72 hr following the instillation of polystyrene microspheres (3.7 mg). The small arrow points to a particle-containing alveolar macrophage (AM) that has engulfed a particle-containing polymorphonuclear leukocyte (PMN).

explanations have been proposed over the last several decades. One general notion has been that the AM are transported passively with alveolar fluids to the ciliated airways, with the propulsive force(s) driving the flow of alveolar fluid being variously related to alveolar fluid dynamics alone or in conjunction with ventilatory excursions, to ventilatory excursions only, and to the more proximal action of ciliary activity (*90,94–100*). Direct experimental evidence that AM may be passively transported by alveolar fluid currents, however, is nonexistent, and the general concept continues to be questioned (*100,101*). As pointed out by Brain and co-workers (*100*), AM appear to be in close apposition with and adherent to the alveolar epithelial surface (*102*), where they are covered by the alveolar lining fluid (*1*). Movement of alveolar fluids, accordingly, seemingly would occur above the AM and exert only limited force to mobilize them. Studies in which the rates of clearance of particles from the lung have been examined under conditions in which ventilatory excursions were increased by exercise or CO_2 inhalation have yielded mixed results (*103,104*). Numerous other factors, such as the aging of AM, however, conceivably could influence their ability to remain adherent to the alveolar surface and their susceptibility to being mobilized by alveolar fluid flows, if such fluid flows indeed occur. As discussed in greater detail elsewhere (*91*), macrophages on the surfaces of the conducting airways are larger and smoother than typical AM, and they generally appear to be an effete stage of the AM. While the effete phenotype of the airway intraluminal macrophages (AI-LM) may have numerous bases, including conditions encountered by the AM once in the airways, the possibility exists that the typical AI-LM phenotype may represent maturation processes that AM undergo while still in the alveoli, and that features associated with effeteness in some manner preferentially facilitate their translocation from the peripheral air spaces. On the other hand, the observation that some AI-LM are undergoing mitosis in the conducting airways (*90*) suggests that not all of this anatomically located subpopulation of pulmonary macrophages are necessarily excessively aged, at least reproductively.

Two other potential mechanisms by which AM may encounter the ciliated airways are related to an ameboid movement of the AM on alveolar surfaces. According to one of these postulates, the AM gain access to the ciliated terminal airways by chance encounters as they randomly migrate along alveolar surfaces. According to the second postulate, the AM gain access to the mucociliary apparatus by a directed ameboid migration of the AM in response to a chemotactic gradient. Either or both of these mechanisms, if operable, would be expected to behave as a first-

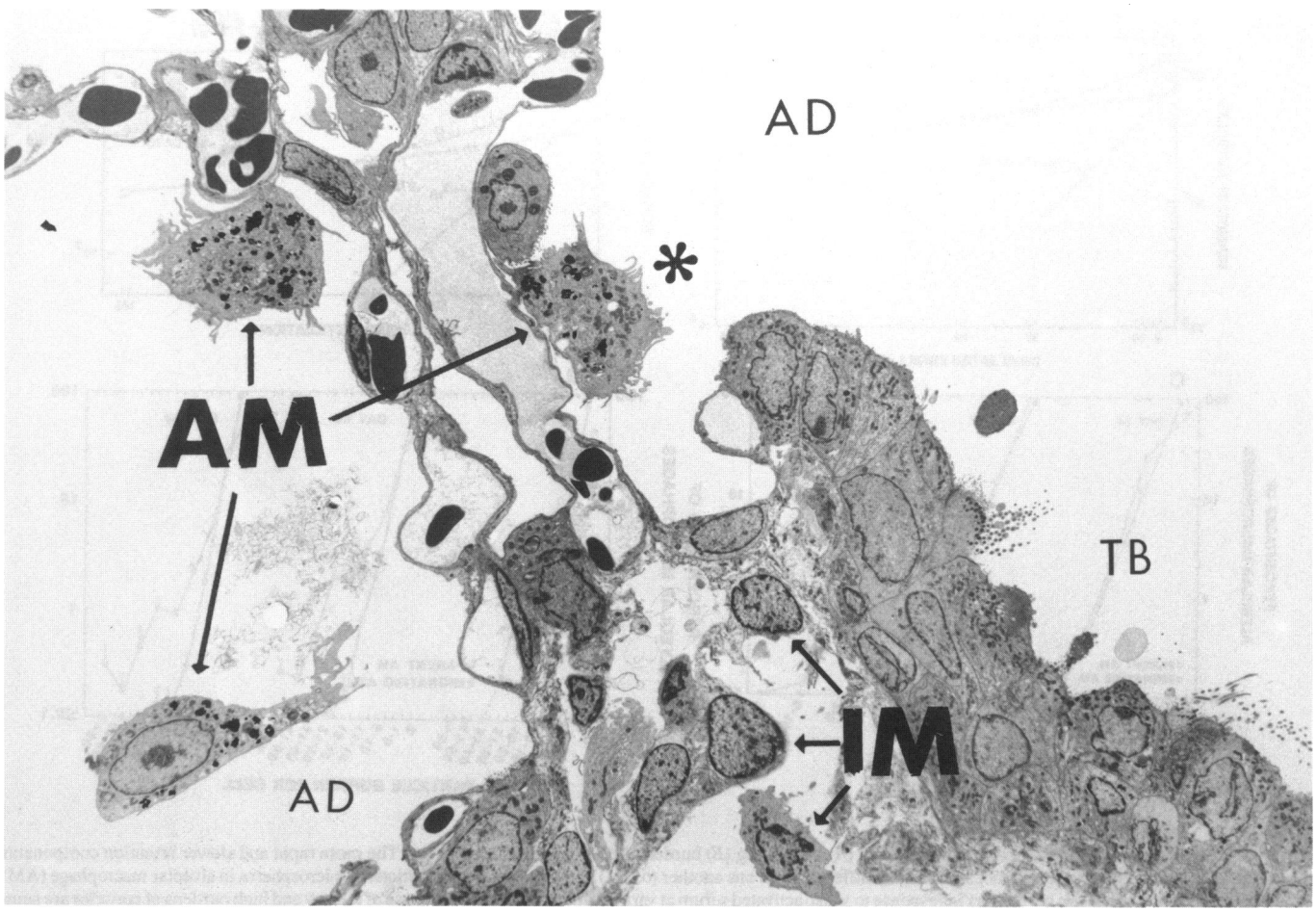

FIGURE 3. Transmission electron micrograph of the alveolar duct (AD)–terminal bronchiolar region. (*) A particle-containing (TiO_2) alveolar macrophage (AM) is near the terminal bronchiole (TB). Some interstitial macrophages (IM) are also present in the alveolar duct–terminal bronchiolar interstitial region. This micrograph was obtained from the lung of a rat after a 90-day exposure to aerosolized, ultra-fine TiO_2 (~ 20 mg/m^3, Degussa, 6 hr/day, 5 days/week).

order process resembling first-order mathematical depictions of the kinetics of alveolar phase clearance (*6,63,64,101,105*). AM do demonstrate random mobility and directed migration in response to a variety of chemotactic factors *in vitro* (*63,107*). Moreover, particles that generate chemotactic factor(s) upon interaction with serum have been shown to cause a local recruitment of AM to the sites where they are deposited (*57,58*), as previously discussed. However, actual observations of AM migration from the alveoli onto the ciliated airways have not been made, and evidence that a chemoattractant is operable in the alveolar–peripheral airway region during alveolar phase clearance is lacking. Nevertheless, several investigators have recently related diminutions in the rate of particle clearance from the lung during a condition of particle overload to a decrease in the mobility of particle-laden AM (*101,108–110*). While *in vitro* studies (*63*) have indeed indicated that the random and chemotactically stimulated migratory activities of AM are increasingly diminished as the load of particles they contain are increased, Lehnert and co-workers (*63*) found no consistent correlation between such particle burden-associated reductions in AM migration *in vitro* and the *in vivo* rates of alveolar clearance of low to higher lung burdens of noncytotoxic particles (Fig. 4). The results of this latter study, however, are complicated by the possibility that the filters used to assess AM migration *in vitro* may have physically restricted the passage of particle-engorged AM. Other issues relevant to the cephalad transport of AM also have yet to be resolved. For example, Green (*99*) has speculated that the passage of AM through the pores of Kohn may serve as a shortcut route by which AM in distal alveoli may become more favorably positioned near the ciliated airways for subsequent removal. This intriguing idea is worthy of experimental consideration.

Whether or not some AM that have engulfed particles in the alveoli may also migrate across the alveolar epithelial lining into interstitial sites as another alveolar surface clearance pathway remains controversial. Both some earlier and some current investigators have suggested that particles enter the alveolar interstitium by the transepithelial migration of AM that phagocytized the particles while in the alveoli (*48,111*). Other investigators have maintained that once phagocytes assume residency in the alveolar space compartment, they do not migrate across the alveolar epithelial cell lining back into interstitial sites (*77,100,112*). Evidence for this latter view largely stems from reported failures

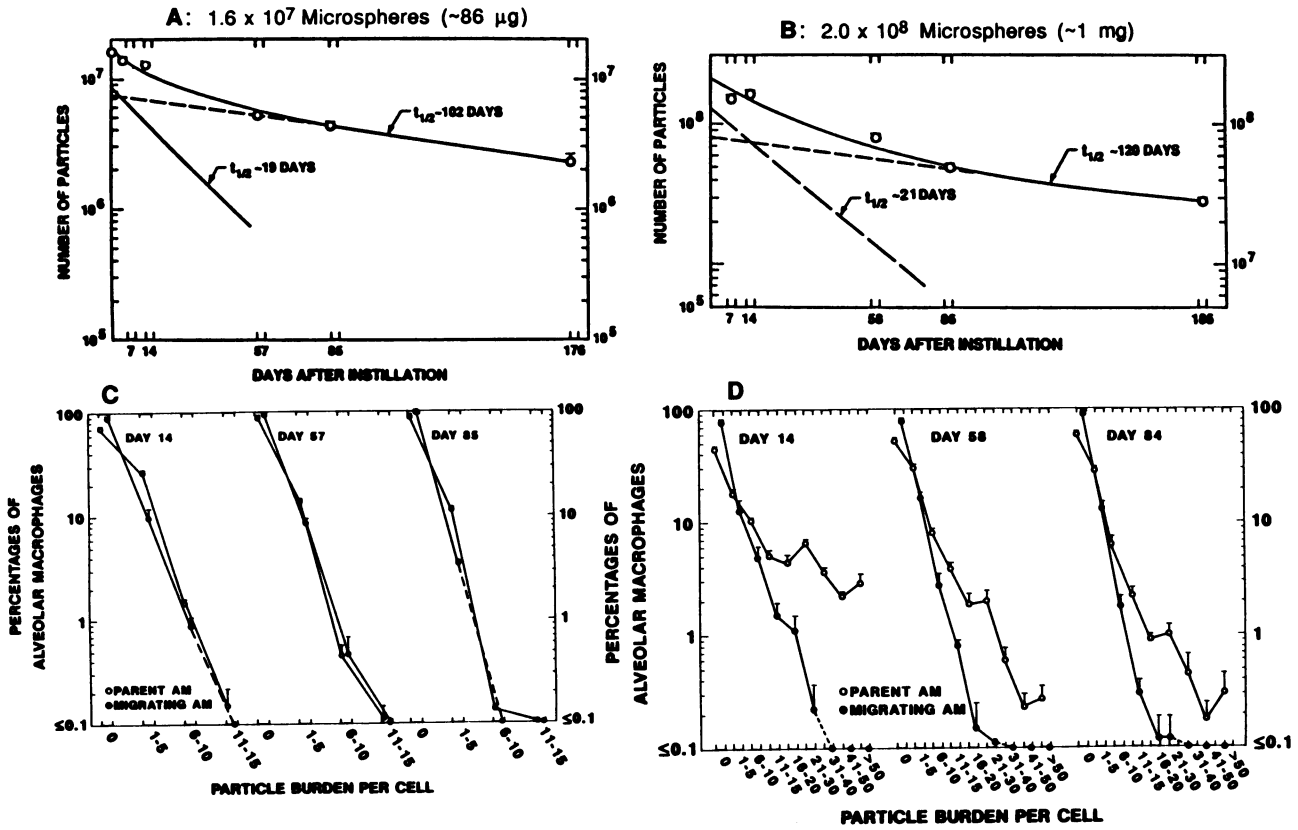

FIGURE 4. Lung retention characteristics of an 86-μg (*A*) and a 1-mg (*B*) burden of polystyrene microspheres. The more rapid and slower retention components of the lower and higher burden are not significantly different from one another (*63,64*). The frequency distributions of microspheres in alveolar macrophage (AM) that migrated through 5-μm pore filters in response to yeast-activated serum at various times during the clearance of the low and high burdens of particles are summarized in *C* and *D*, respectively (*63*). The particle distributions in parent AM populations lavaged from the lungs that received the low burden (*C*) were similar to those of the AM from these lungs that migrated across the filters. On the other hand, AM with high particle loads in lavage samples obtained from lungs instilled with the high burden of particles (*D*) showed diminished abilities to migrate across the filters in response chemotactic stimulation.

to find ultrastructural support for such a cell-translocating mechanism and from interpretations that macrophages observed by electron microscopy to be migrating between alveolar epithelial cells were interstitial macrophages (IM) translocating into the alveoli as opposed to AM migrating into the interstitium (*77,81,113*). The difficulty in determining the origin and direction of macrophages migrating across the alveolar epithelial barrier is illustrated by Figure 5. In this micrograph, a small extention of a macrophage present in an alveolus extends between two alveolar epithelial cells. The phagocytized lamellar material in the macrophage suggests that it may be an AM in the process of undergoing passage into the interstitial compartment. As an alternative interpretation, the macrophage may also be an IM approaching a final stage of passage from the interstitium into an alveolus where it encountered and engulfed lamellar material.

Other lines of experimental evidence regarding the passage of AM into the interstitium (where they would become interstitial macrophages by anatomical definition) are also equivocal. Following the aerosol exposure of rabbits to metallic nickel dust, Johansson and Camner (*114*) observed some macrophages containing particles and laminated bodies in the hilar lymph nodes. These lymph nodal macrophages were considered to be similar in appearance to AM in the alveoli. How the state of phospholipidosis and other pulmonary abnormalities induced by the nickel dust may have figured into this observation is unclear.

In another study, Corry and co-workers (*115*) intratracheally instilled radiolabeled AM into syngeneic guinea pigs and found radioactivity in the hilar lymph nodes as well as radiolabeled cells in autoradiographic preparations of regional lymph nodal cell suspensions obtained 1–3 days after the instillations. Some procedural aspects of this study, however, preclude a precise interpretation of these outcomes (*116*). Lehnert and co-workers (*116*) also examined mononuclear phagocytes from the tracheobronchial lymph nodes of rats for the presence of particles after the intratracheal instillation of noncytotoxic polystyrene microspheres. A discordance was found between the frequency distributions of the microspheres in the nodal macrophages over a 30-day period after particle deposition relative to the frequency distributions of the particles in AM lavaged over the same duration, with the nodal phagocytes containing lower cellular burdens of the particles than did the AM (Fig. 6). These latter findings suggest that if AM do translocate across the alveolar epithelial barrier in the rat's lung, the process may be restricted by the magnitude of the particulate burdens they contain. In this same study, most of the particles that reached the tracheobronchial lymph nodes were not contained in phagocytes (Fig. 7) which,

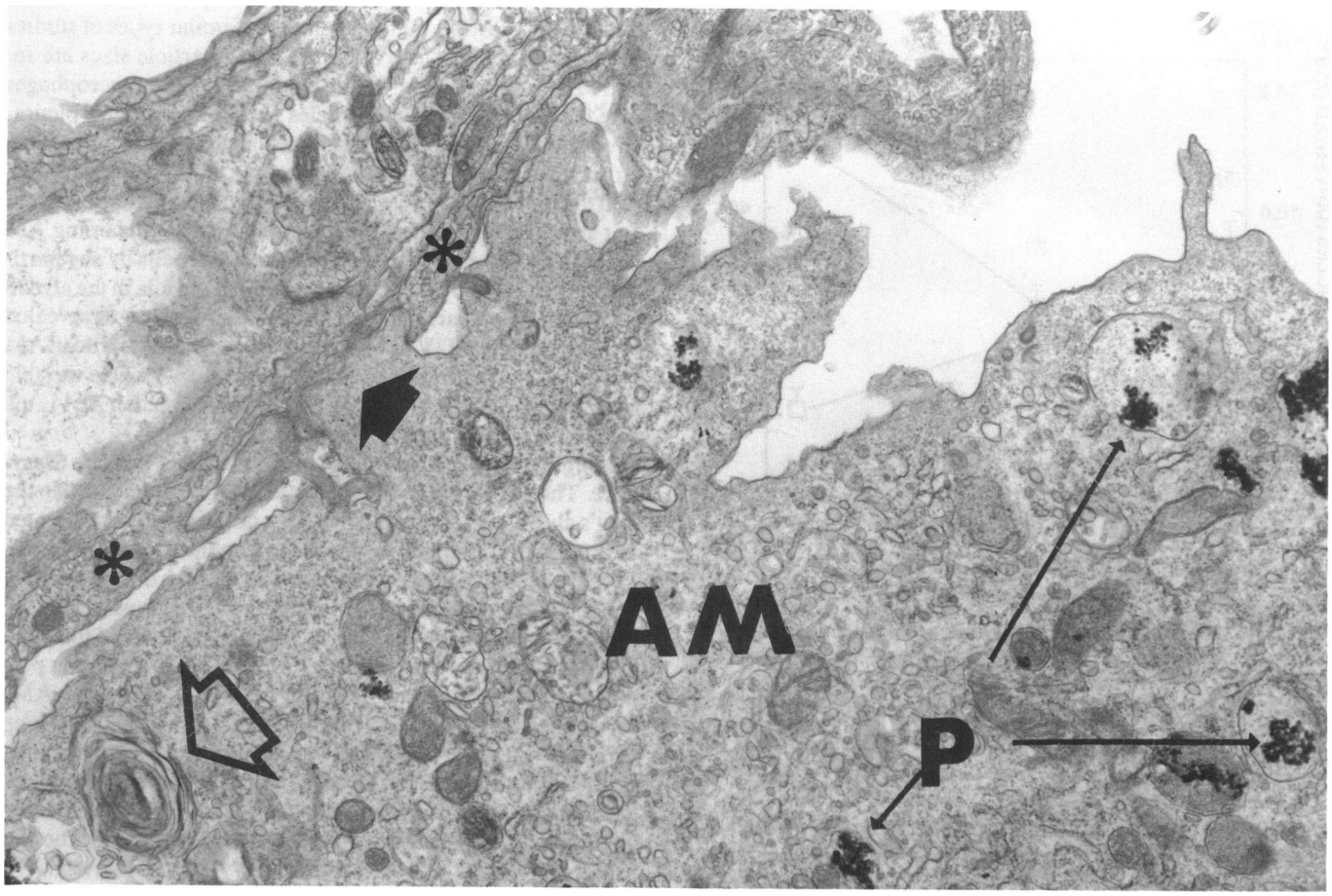

FIGURE 5. Transmission electron micrograph of a macrophage that appears to be either entering the interstitial compartment or that is finalizing its entry into an alveolus from the interstitial compartment. Because the macrophage is predominantly in an alveolus, it is labeled as an alveolar macrophage (AM). (P) Particulate TiO_2; (open arrow) engulfed lamellar material; (solid arrow) projection of the macrophage between two type I epithelial cells (*). The basal lamina at the base of the projection is indistinct. This micrograph is of the lung of a rat that was exposed to aerosolized Degussa TiO_2 over a 90-day period.

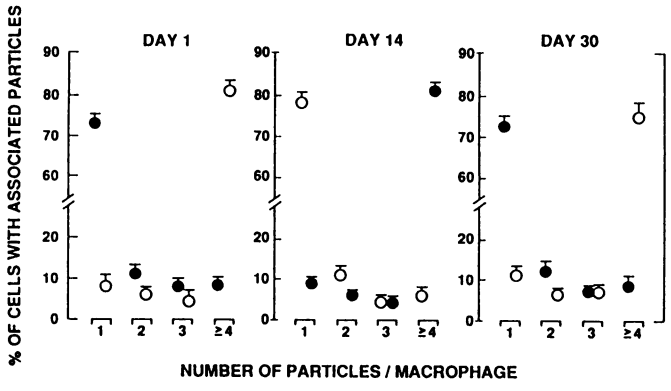

FIGURE 6. Lungs of rats were intratracheally instilled with 4×10^8 polystyrene microspheres and the frequency distributions of the particles in alveolar macrophages (AM), and nodal macrophages that contained them were determined over a 30-day period. (○) Lavaged AM; (●) lymph nodal mononuclear phagocytes. No nodal macrophages in this study contained more than eight microspheres, whereas more than 20% of the AM contained >20 particles at all assay times (*116*).

consistent with the observations of several other investigators (*117,118*), further suggested that the majority of particles that were translocated to the lymph nodes arrived there as free particles and not in AM that may have entered the lung's interstitium.

Lee and co-workers (*48*), on the other hand, reported evidence that only small numbers of free particles were in the lymphatic channels in the lungs of rats that received relatively high burdens of TiO_2 by chronic aerosol exposure, and that most of the particles in the channels and tracheobronchial lymph nodes were in macrophages, many of which morphologically appeared to be AM. Suggestive evidence that the migration of both particle-containing AM and PMN may contribute to the passage of particles into the interstitial compartment and to the subsequent translocation of particles to draining lymph nodes has also been obtained in the dog model (*119,120*). How the intrabronchial instillation techniques and other protocols used in these dog studies may have affected the results, however, are unknown. Moreover, the conclusions drawn in these studies would have gained further support if comparisons of the frequency distributions of particles in the lung free-cells and lung lymph nodes had been shown to be similar. Nevertheless, the above studies collectively seem to suggest that particle-containing AM may indeed make their way

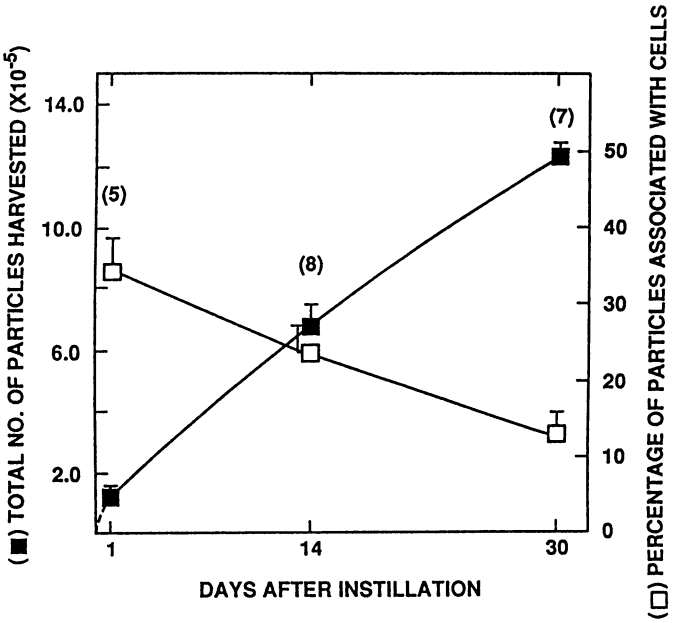

FIGURE 7. (■) Total numbers of particles in the tracheobronchial lymph nodes on days 1, 14, and 30 after the instillations of the polystyrene microspheres (*116*). (□) Percentages of the nodal particles that were associated with nodal macrophages. Numbers in parentheses are numbers of animals examined at each time point.

into the lung's interstitium and subsequently enter lymphatic channels for subsequent transport to the regional nodes under at least some perhaps extraordinary or special conditions, but how the above findings may extend to the human condition has not been investigated. Conceivably, this pathway may be more or less operational as functions of the mammalian species involved, the types and burdens of particles that are deposited in the alveoli, the rate at which particles are deposited, and the time period after which the particles are deposited. Indirect support of the idea that different mechanisms may be operable in different species, at least, comes from observations that the rate of transport of particles from the alveolar region to the lung-associated lymph nodes can van considerably among species (*121,122*).

The possibility that AM with particles may also migrate across the visceral pleura into the pleural space as another lung clearance pathway has received little experimental attention. Such a pathway is suggested by observations that some types of inhaled particles, e.g., asbestos fibers, can gain access to the visceral and parietal pleurae and the pleural space compartment, and that particles that have reached the pleural space can be contained in macrophages (*123–126*). In a preliminary study of the translocation of particles from the alveoli into the pleural space compartment, Hyler et al. (*127*) intratracheally instilled polystyrene microspheres into the lungs of rats, lavaged the pleural space and lungs separately, and indeed found particle-containing macrophages with morphologic features similar to AM among the retrieved pleural cells as early as 1 day after the instillations. In a subsequent study, however, Lehnert and colleagues (*128*) determined that lavage of the pleural cavity can also harvest AM from the lung, and, by using a modified pleural wash procedure that reduced lung trauma, that microspheres with a diameter of about 2 μm are not translocated to the pleural space even when intratracheally instilled at high numbers. Similar types of studies involving different types of particles and particle sizes are required to assess the role AM or other pulmonary macrophages may have in transporting particles across the visceral pleura. Even if AM do not normally migrate into the pleural space, they probably can be introduced into the pleural space compartment with the particles they may contain during active pleural effusion.

It should be pointed out that not all particle-containing AM necessarily leave the alveolar space as intact cells by any particular route. Some AM apparently simply die while in the alveoli and release their particles onto the alveolar surface, as revealed by Heppleston (*129,130*), whereas some other effete or dead AM may be phagocytized by more healthy AM (Fig. 8). One variable that likely affects the mortality rate of particle-laden AM is the cytotoxicity of the particles they contain.

Lung Retention Kinetics and AM-Associated Particle Clearance. The retention characteristics for particles deposited in the lung's alveolar region have often been descriptively represented as a multicompartment or multicomponent process (*105,106, 131,132*), although the physiologic bases for such depictions have not been well characterized. Inasmuch as the translocation of particle-laden AM up the conducting airways is held to be the predominant process involved in the removal of insoluble particles from the lung, we recently investigated lavaged AM-particle relationships during the alveolar clearance of a low lung burden ($\sim$86 μg) of polystyrene microspheres ($\sim$2 μm diameter) as one approach to obtain information on potential AM-related mechanisms that could form the underlying bases for conventional depictions of alveolar clearance (*132*). Briefly, the apparent overall rate(s) of disappearance of AM from the total AM population over time was found to increase with increasing particle burdens contained in the AM (Fig. 9), and, for cellular burdens up to $\sim$14 microspheres, the disappearance of AM from the total AM population followed a pattern, which like the lung retention data (Fig. 4*A*), could be described by a two-component, negative exponential equation for each AM-particle burden category. AM with higher burdens disappeared from the AM population monoexponentially. Thus, some evidence was obtained that the overall lung retention characteristics of the particles had a qualitative AM counterpart. Additionally, this study also demonstrated that an increasing fraction of the particles that were retained in the lung over time was contained in AM with the lowest particle burdens (Fig. 10).

We previously suggested (*132*) that the findings summarized in Figures 9 and 10 were *a*) consistent with a preferential, enhanced rate of removal of AM via the tracheobronchial route as their cellular burdens of particles increase, and/or *b*) that particles are gradually redistributed among the lung's AM population over the course of alveolar phase clearance concurrent with the removal of particle containing AM via the conducting airways (*132*). Our more recent findings that the frequency distributions of particles in AI-LM and AM are virtually identical over the course of alveolar phase clearance (*91*) (Fig. 11), as well as observations made in other studies that the absolute numbers of AM with lower burdens of microspheres can actually increase during alveolar clearance (*64*), has provided further support for the latter possibility. Though all of the mechanisms by which particles are redistributed among the lung's AM concurrent with the

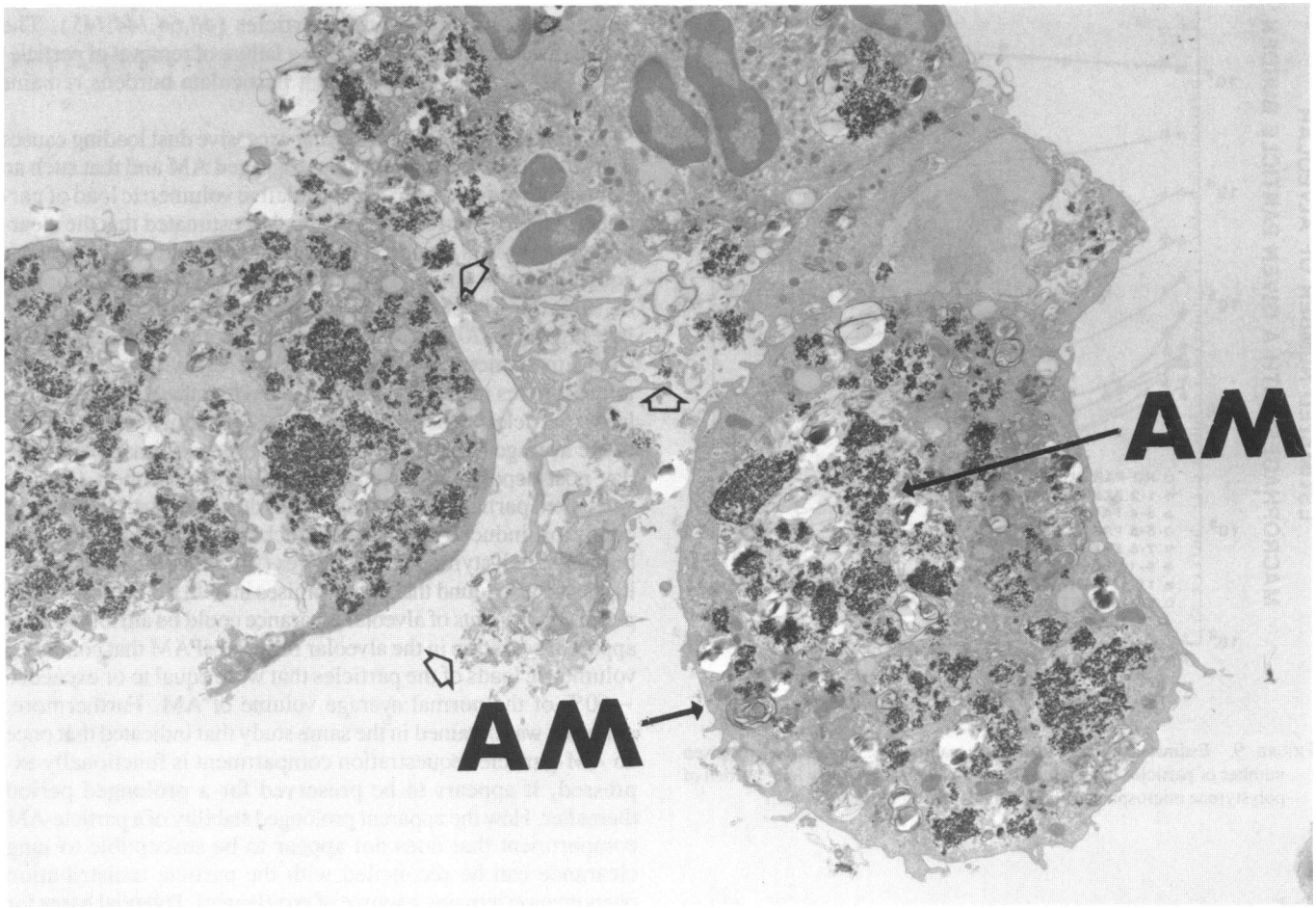

FIGURE 8. Transmission electron micrograph showing a particle-containing alveolar macrophage (AM) (short arrow) that has been engulfed by another AM (long arrow) in an alveolus in the lung of a rat that was exposed over a 90-day period to aerosolized TiO_2 (Degussa). The open arrows point to free particles that have been released upon the autolysis of a particle-containing phagocyte.

physical transport of AM from the lower respiratory tract remain to be determined, they could include *a*) the *in situ* autolysis of particle-containing AM and the re-uptake of particles by other AM with preexisting or no particle burdens, *b*) the *in situ* division of particle-containing AM and allocations of the parent AM's particles to progeny cells, *c*) the phagocytosis of effete particle-containing AM by other AM, *d*) the direct transfer of particles from one AM to another AM, and *e*) the exocytosis of particles by AM and the uptake of particles by other AM. Experimental support for at least some of these potential processes has been obtained in a variety of investigations involving AM and other endocytic cell types (*79,129,130,133–136*). The effect that particle redistributions among the lung's AM may have on the retention of particles in the lung remains to be examined further. Assuming, as our analyses of particle burdens in AI-LM and AM have suggested, that the removal of AM from the lung by mucociliary transport is independent of their cellular burdens, and, if the alveolar removal of AM is otherwise stochastic and is a first-order process, the "particle redistribution phenomenon" may have no significant influence on the kinetics of particle retention in the lung. The same number of particles in AM would be expected to be removed from the lung per unit time whether 30% of the cells in the total AM population contained 10 particles per cell or 60% of the AM population contained only 5 particles per cell. On the other hand, if the particle redistribution phenomenon is predominantly due to process(es) that involve the release of free particles onto the alveolar surface, some released particles potentially could gain access into extra-AM compartments, i.e., type I pneumocytes and the interstitium, where their retention may be relatively more prolonged.

AM-Associated Particle Clearance and the Particle Overload Phenomenon. A special condition that is currently receiving substantial attention is the phenomenon of particle "overload." This condition, which is hallmarked by lung burden-dependent diminutions of normal lung clearance rates, has been observed following the deposition of high lung burdens of virtually all types of relatively insoluble particles examined to date, including titanium dioxide, particulate diesel exhaust, chromium dioxide, carbon black, and polystyrene microspheres (*64,137–144*). A compromise in AM-associated particle clearance is likely a predominant cause of diminished lung clearance rates during particle overload inasmuch as the AM are the primary reservoirs for particle containment in such a condition. Histologically, clusters or aggregates of particle-laden AM are typically ob-

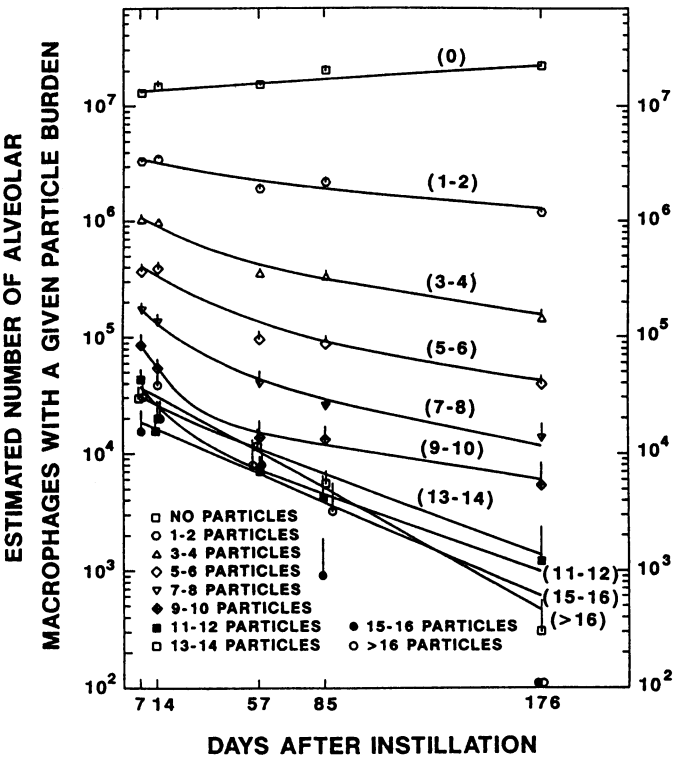

FIGURE 9. Estimated numbers of alveolar macrophages that contained a given number of particles during the alveolar clearance of a low lung burden of polystyrene microspheres (*132*). Values represent means + SE.

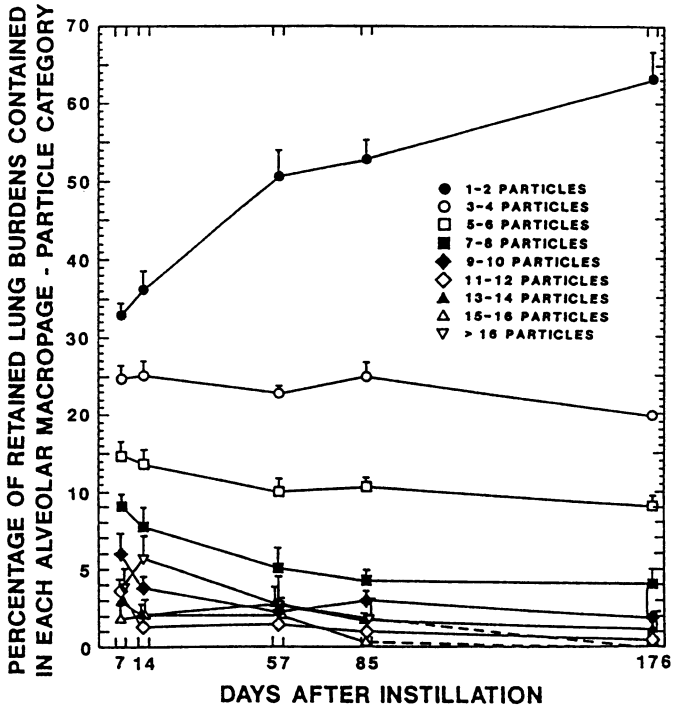

FIGURE 10. Over time, the percentage of a retained lung burden of polystyrene microspheres was found to be progressively contained in alveolar macrophages with low particle loads (*132*).

served in the alveoli of animals that have been exposed to high concentrations of insoluble particles (*48,64,144,145*). The actual mechanism(s) that underlie a failure of removal of particle-laden AM from lungs with high particulate burdens remains obscure.

Morrow (*101*) has proposed that excessive dust loading causes a loss in the mobility of particle-engorged AM and that such an impediment is related to the cumulative volumetric load of particles in the AM. Morrow (*101*) further estimated that the clearance of an AM is impaired when the particle load it contains is equivalent to ~60% of its normal volume. Evidence in support of Morrow's hypothesis is mounting. Oberdorster and co-investigators (*110*) assessed the alveolar clearance of smaller (~3-μm diameter) and larger (~10-μm diameter) polystyrene microspheres in the rat, and they found that the clearance of the larger particles, which volumetrically were equivalent to ~60% of the average volume of normal AM, was minimal over a 200 day post-deposition period. More recently, Lehnert (*64*) investigated particle-AM relationships during a particle overload condition induced by the intratracheal instillation of a high burden of polystyrene microspheres (~2-μm diameter) into rat lungs and also found that compromises in both the early and later phase components of alveolar clearance could be attributed to an apparent cessation in the alveolar removal of AM that contained volumetric loads of the particles that were equal to or exceeded ~60% of the normal average volume of AM. Furthermore, evidence was obtained in the same study that indicated that once an AM-particle sequestration compartment is functionally expressed, it appears to be preserved for a prolonged period thereafter. How the apparent prolonged stability of a particle-AM compartment that does not appear to be susceptible to lung clearance can be reconciled with the particle redistribution phenomenon remains a source of puzzlement. Potential bases for the appearance of aggregated AM in the alveoli of lungs overloaded with particles have been discussed elsewhere (*146*).

Macrophages in Conducting Airways

Intraluminal Macrophages. Macrophages are present on the intraluminal surfaces of both large- and small-caliber airways (*147*) (Fig. 12). These airway intraluminal macrophages (AI-LM) are histologically best studied *in situ* when the lung tissue is fixed by vascular perfusion. The introduction of fixative directly into the airways mobilizes substantial numbers of these macrophages from their original airway locations (*147*). In well-prepared samples, the AI-LM are found on the mucous lining of the airways, submersed in the mucous lining, or often present beneath the mucous lining in close apposition with underlying airway epithelial cells (*90,146,147*). The numeric abundance of AI-LM along the conducting airways and their abundance relative to other macrophage subpopulations in the lower respiratory airways have not been well established and may be species dependent.

Warheit and co-workers (*148*) harvested AI-LM by lavaging the trachea and extrapulmonary main stem bronchi of the rat and compared the numbers of macrophages obtained from the airways with the numbers of more peripherally located alveolar macrophages (AM) that were harvested by a less-than-exhaustive bronchoalveolar lavage technique. Based on recovered cell numbers, these investigators estimated that the tracheobronchial

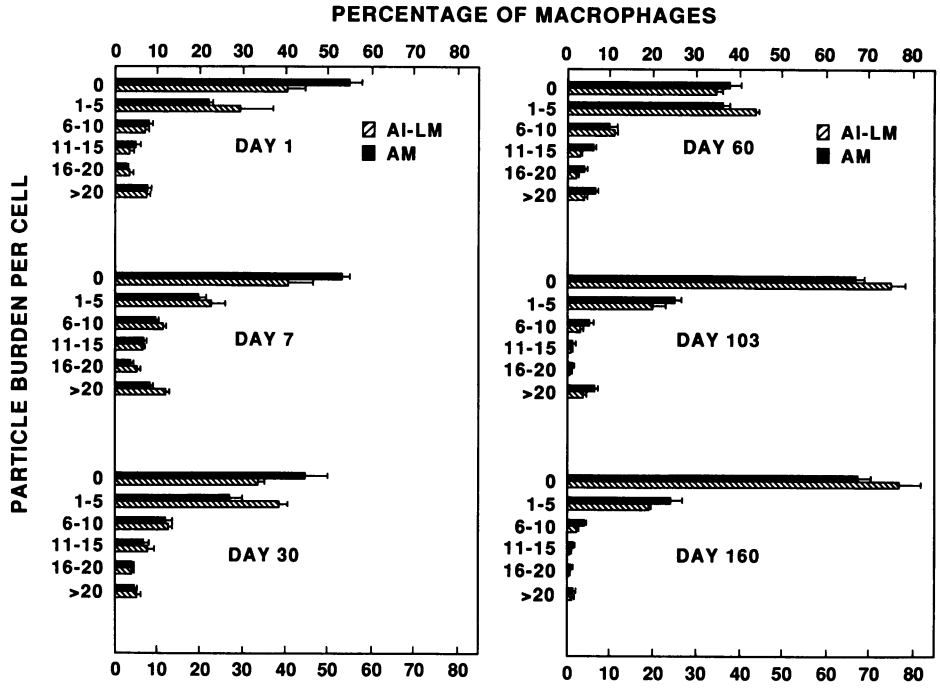

FIGURE 11. Comparisons of the frequency distributions of particles in tracheal airway intraluminal macrophages (AI-LM) and alveolar macrophages (AM) at various times following the instillation of 1 mg of polystyrene microspheres into the lungs of rats. At all times after instillation, the distributions of particles in the AI-LM and AM were similar.

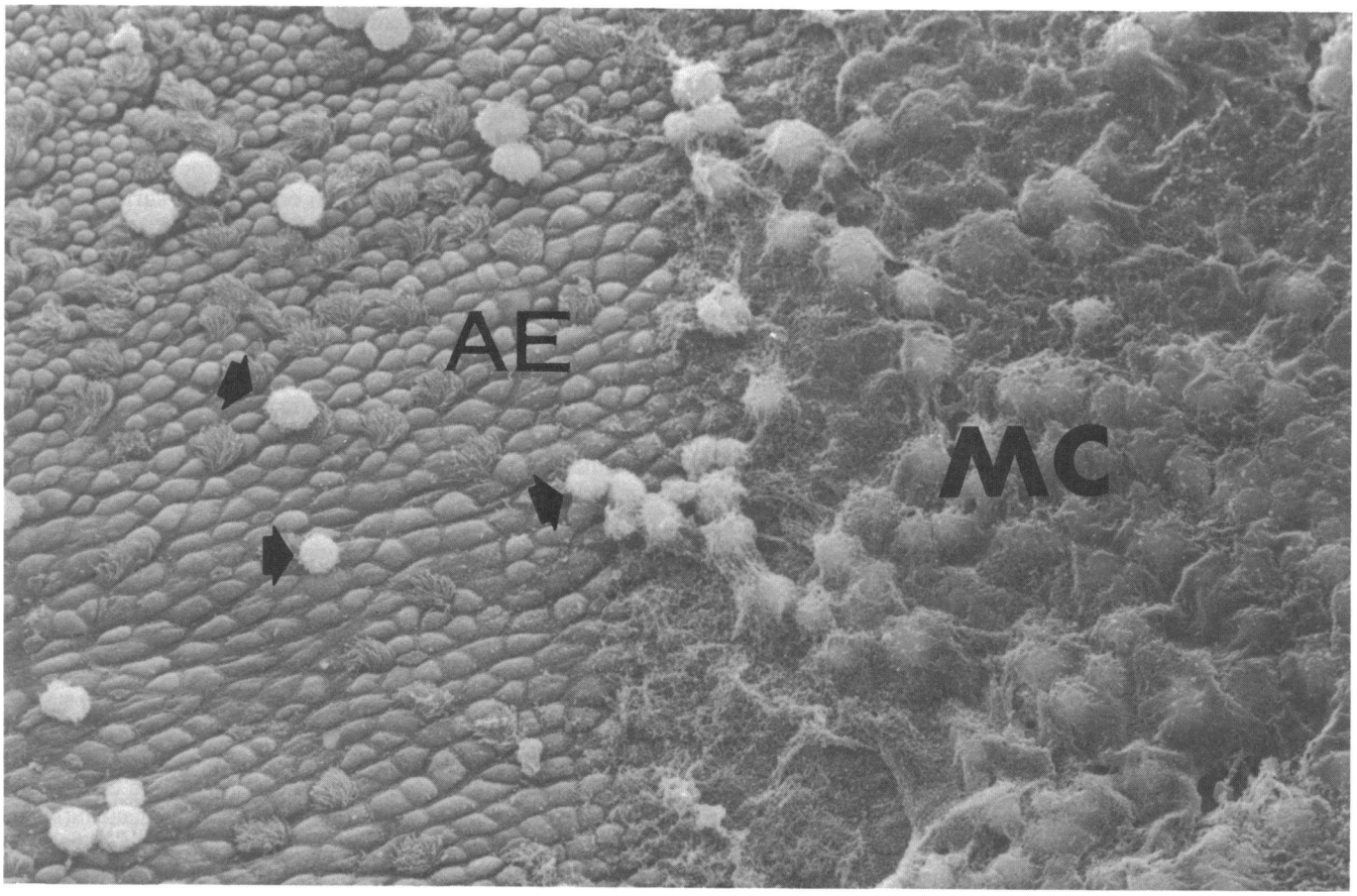

FIGURE 12. Scanning electron micrograph of the luminal surface of a rat's trachea. (AE) airway epithelial surface; (MC) mucus covering the airway surface. (Arrows) intraluminal free cells, some or all of which are airway intraluminal macrophages.

AI-LM ($\sim 6 \times 10^4$ cells) accounted for 5–8% of all the macrophages obtained by conventional lavage. Similar to the findings of Warheit et al. (*148*), Lehnert and co-investigators (*91*) found that approximately 3×10^4 AI-LM could be recovered from the trachea and extrahilar main stem bronchi of SPF rats, and they estimated that, on average, one AI-LM exists per approximately 1×10^4 μm^2 luminal surface area of the airways sampled (*149*). In the alveolar region of the rat's lung where the number of AM is closely similar to the number of alveoli (*10,61*), an average of one AM exists per approximately 2×10^4 μm^2 alveolar surface area. Accordingly, even though the Al-LM can be estimated to account for only about 2–3% of all of the lung's supra-epithelial macrophages (*6,10*), by extrapolating the relationship of the number of AI-LM per unit surface area in the trachea and main stem bronchi to the surface area of all the conducting airways in the rat (*149*), the relative abundance of the AI-LM may be similar to that of AM in the alveoli on a surface area basis. The results of these considerations of the relative availability of AI-LM along the conducting airways suggest that this subpopulation of pulmonary macrophages could play important functional roles on the airway surface like those numerous functional activities that have been attributed to the AM in the alveoli (*2–7*). It should be noted that with a less-than-extensive lavage technique, the fractional contamination of lavaged cells with AI-LM may be of experimental importance. On the other hand, the relative contribution the AI-LM make to the total numbers of lavaged cells should decline when the more distal air spaces are more completely sampled. With regard to potential species differences in the occurrence of AI-LM, we have been unable to recover more than a few AI-LM from the tracheas of ferrets, even when the mucolytic *N*-acetylcysteine was used in the wash fluid to facilitate their removal (unpublished observations).

Unlike the well-studied AM, the AI-LM have only recently received experimental attention. Compared to the pulmonary macrophages obatined by conventional bronchoalveolar lavage, which presumably consist mainly of alveolar macrophages, the tracheobronchial macrophages in the rat are composed of a greater percentage of cells with smoother surfaces than are the more ruffled AM (*91,148*), and the AI-LM are generally larger than AM as well (*91*). Also, the abilities of the AI-LM to exclude trypan blue and their abilities to phagocytize IgG-coated particles and carbonyl particles *in vitro* are less than those observed with macrophages obtained by bronchoalveolar lavage (*91,148,150*). Recent comparative analyses of flow cytometrically sorted AI-LM and AM for the lysosomal enzymes acid phosphatase, nonspecific esterase, and β-glucuronidase have also indicated that the activities of these enzymes in the AI-LM are less than in the AM (*91*). These findings, as well as others (*91*), have led us to postulate that the AI-LM are effete relative to their AM precursor form, as previously mentioned.

Several investigators have postulated that the AI-LM in the healthy lung are AM that are undergoing cephalad removal via mucociliary transport (*90,147,150–152*). More recently, Lehnert and co-workers (*91*) reported that AI-LM and AM have similar flow cytometrically resolvable electro-optical phenotypes, and these investigators determined that the AI-LM label for an antigen present on AM that is identifiable using the monoclonal antibody ED1 (*153*). Neither the AI-LM nor the AM were found to label for a surface antigen present on pulmonary perivascular and peribronchial macrophages that is identified by the monoclonal antibody ED2 (*153,154*). Further evidence of an AM origin for the AI-LM comes from comparisons of the frequency distributions of particles in rat AI-LM and AM harvested from the same lungs during the alveolar clearance of an initially instilled lung burden of polystyrene microspheres (2×10^8, ~ 2-μm diameter). As previously indicated (Fig. 11), both the percentages of the AI-LM and AM that contained the test particles and the distributions of the microspheres among the AI-LM and AM were closely similar over a 160-day period after the particles were deposited; as a point of reference, the retention characteristics of the lung burden of microspheres used (*63*) in this study are illustrated in Figure 4*B*. Thus, the collective evidence to date indicates that the AI-LM do indeed originate as AM, at least in the healthy rat lung.

The AM, however, may not be the exclusive source of the AI-LM under some conditions. Bowden and Adamson (*38*), for example, found evidence of a migration of blood monocytes across the bronchiolar epithelium within 1–2 days after the instillation of carbon particles into the lungs of mice, and similar cell recruitments of macrophages have been reported in the rabbit during the "late asthmatic response" (*155*). Moreover, Hulbert et al. (*156*) observed macrophages migrating through guinea pig tracheal epithelium with PMN in response to cigarette smoke. Both cell types were found to migrate in file through distinct pathways, or "solitary openings," rather than randomly between the airway epithelial cells. Similar migrations of macrophages onto the luminal surfaces of the conducting airways may have accounted for Brundelet's (*157*) previous conclusion derived from studies of diseased rat lungs that an ultimate clearance pathway for alveolar macrophages involves their passage across the surface of bronchial lumina. Tucker and co-investigators (*158*) also contended that pulmonary interstitial macrophages translocate across the bronchial epithelium onto airway surfaces, but as pointed out by other investigators (*90,100*), the histologic techniques used in their study did not provide the necessary resolving power to unequivocally demonstrate this migratory pathway. We, as well as other investigators (*159,160*), have found no electron microscopic evidence to date in support of a migration of macrophagelike cells across the airway epithelial barrier of the conducting airways of rats. Additionally, this migratory pathway does not appear to be significantly operable in the absence of an inflammatory response in the mouse (*90*).

Information about the residency times of AI-LM in the conducting airways has yet to be obtained. Estimates of the numbers of macrophages that are removed from the lung daily have a broad range. Data obtained by Lehnert and Morrow (*6*) and by Shellito and co-workers (*45*) suggest approximately 1–2% of the rat's resident AM population is translocated from the lung every 24 hr, presumably as AI-LM. On the other hand, the results of a study performed by Spritzer and co-investigators (*161*) suggest that as many as approximately 2.4×10^7 macrophages leave the adult rat's lung on a daily basis, which is essentially equivalent to the total number of AM present at any time in that species (*6,10*), during steady-state conditions. The mechanism(s) and corresponding rate(s) for the transport of AI-LM up the conducting airways remain to be determined. Conceivably, the cephalad transport of the AI-LM may be accomplished passively under the direct influence of transport rates of local mucus. However, con-

sistent with histologic interpretations that the residency times of at least some of the AI-LM along the airways may be prolonged (*90*), considerations of this matter suggests that not all available AI-LM may be so readily removed. Given a rat tracheal length of approximately 27 mm, an average tracheal rate of mucous clearance of approximately 5 mm/min (*162*), and approximately 3×10^4 AI-LM in a normal rat's trachea at any time, approximately 3×10^5 AI-LM should leave the trachea per hour if the AI-LM simply are removed passively from the trachea by mucous currents. This hourly value translates into approximately 8×10^6 cells per day, or the equivalent of the removal of approximately 30% of the resident AM population (*10*). While this seemingly high rate of removal is directionally consistent with that reported by Spritzer et al. (*161*), such a high daily number of translocating macrophages is generally inconsistent with some existing information about mechanisms and corresponding rates by which AM are replenished in rat lungs under homeostatic conditions (*6,45,163*). Yet, we have recently found that AM lavaged from healthy rat lungs can proliferate *in vitro* under suitable culture conditions with a doubling time of about 1 day (*136*). Extending this observation to the *in vivo* condition would imply that the entire AM population in the rat could be replenished daily under suitable conditions merely by the *in situ* proliferation of resident AM.

The AI-LM may play several roles in the removal of insoluble particles from the lungs with one function being the phagocytosis of inhaled particles that deposit on airways. *In vitro*, the AI-LM show both opsonin-dependent and opsonin-independent phagocytic activity (*91,148,150*). In support of a phagocytic role for the AI-LM in the lung, these *in vitro* findings have been recently complemented by the observation that the AI-LM can phagocytize inhaled particles *in situ* shortly after deposition on the conducting airways (*164*). In addition to the phagocytosis of particles that deposit on the airways, another prominent role of the AI-LM population is that of a cellular compartment for the sequestration of particles as they are transported up the mucociliary apparatus from the alveolar region. Even though a substantial fraction of the AI-LM is composed of dead cells (*150*), such a particle sequestering function appears to remain essentially intact as the AI-LM are removed from the lower respiratory tract, i.e., the fidelity of particle containment in the AI-LM is apparently well preserved even when the AI-LM are no longer viable. This conclusion is based on results obtained in our laboratory in which we found few free particles in tracheal lavage samples in our analyses of particle burdens in AI-LM and AM during the alveolar clearance of instilled microspheres. Whether the AI-LM can release chemotactic factor(s) for the recruitment of additional phagocytes onto the airways following the engulfment of some types of particles, as AM are thought to do in the alveoli (*74*), has not been examined. It is also unknown whether the AI-LM respond to chemotactic gradients as a means to facilitate the efficiency of encountering and phagocytizing particles on airway surfaces. With regard to this latter potential function, however, some indirect evidence suggests that the AI-LM may be less responsive to chemotactic stimuli than are AM (*91*), and, accordingly, the AI-LM may engulf particles mainly upon randomly encountering the particles that deposit in their local vicinity. Regardless, a relatively slow removal of some AI-LM that have phagocytized particles deposited in the conducting airways may provide some explanation for observations that the tracheobronchial clearance of particles is more prolonged (*165–167*) than previously though, assuming that all AI-LM do not simply undergo continuous, passive removal up the tracheobronchial tree under the influence of the mucociliary apparatus.

Information about how the relative abundance of AI-LM may relate to the underlying size of the resident AM population during the alveolar clearance of particles requires further study. If the passage of AM onto the mucociliary apparatus is purely a stochastic process following a pattern of first-order kinetics, it is conceivable that the numbers of AI-LM in the conducting airways may be directly proportional to the number of more peripheral AM. Concurrent with the aforementioned study in which we comparatively examined the frequency distributions of polystyrene microspheres in AI-LM and AM during alveolar phase clearance, we also attempted to gain some insight into AI-LM and AM relationships by additionally enumerating the AI-LM and AM (Fig. 13). Two general observations were made in this component of our study. First, the numbers of AI-LM in the trachea did not directionally scale with the numbers of AM lavaged by a standardized protocol, but, instead, the relative abundance of the AI-LM appeared to be inversely proportional to the underlying size of the AM population. Second, the numbers of AI-LM were considerably lower during the more rapid phase of alveolar clearance than during later phase alveolar clearance, (Fig. 4*B*). While these findings are subject to numerous explanations (and they certainly do not provide sufficient information for a definitive conclusion about how the numbers of AI-LM that are removed from the lower respiratory tract per unit time relate to the AM population size), they do suggest that the rate of removal of AI-LM from the conducting airways may indeed be more rapid during early alveolar phase clearance than during later alveolar phase clearance.

Macrophages in the Airway Epithelium. A second anatomically defineable population of airway cells that may be

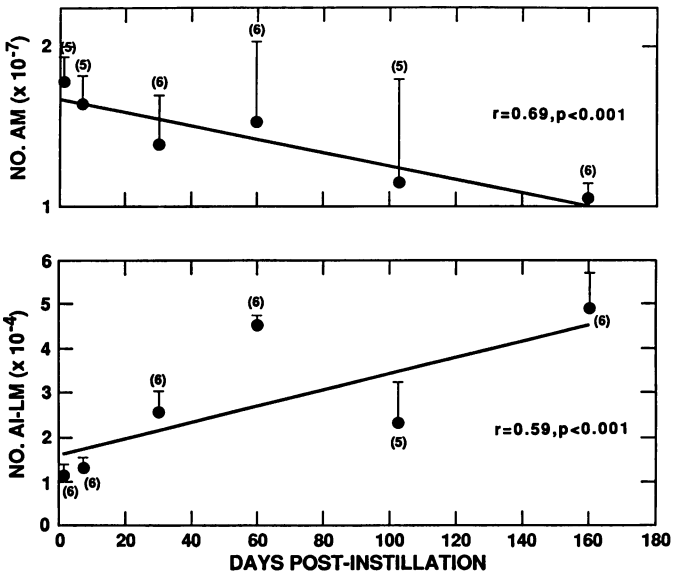

FIGURE 13. Numbers of airway intraluminal macrophages (AI-LM) and alveolar macrophages (AM) separately lavaged from the same trachea–lung samples at various times following intratracheal instillations of 1 mg polystyrene microspheres into the lungs of rats.

related to the mononuclear phagocyte system has also been observed in association with the epithelial lining of the conducting airways. In the mouse, these cells, which morphologically resemble epidermal Langerhans cells and label for the murine macrophage-specific antigen F4/80, are located at the base of the pseudostratified epithelium on the luminal side of the basement membrane (*168*). F4/80-positive cells also occur in association with the epithelial lining of the ducts of the tracheobronchial glands (*168*). Whether Langerhans cells, which are recognized by charateristic intracytoplasmic Bibeck structures (*169*), are present in the conducting airways of all mammalian species remains to be determined, but it is known that they can exist between epithelial cells in the bronchioles of some humans, and their relative abundance may be increased in some diseased states, e.g., idiopathic pulmonary fibrosis (*170*). The Langerhans cells in the airways have as yet to be studied in great detail, but if these cells are typical of Langerhans cells in other tissue sites, they have receptors for the Fc component of IgG and the C3 component of complement, and they are generally poorly phagocytic and have a lower content of lysosomal enzymes than do most other types of macrophages. Another dendritic cell type has also been observed in the airway epithelium of the conducting airways of the human (*171*). While this cell type shares some ultrastructural features common to Langerhans cells, they do not contain Birbeck granules. Current evidence suggests the dendriticlike cells in the lower respiratory tract functionally participate in immune responses with one role being that of accessory cells for the induction of lymphocyte proliferation in response to antigens (*172,173*). Essentially nothing is known about the functional role(s), if any, that these or the Langerhans cells may play following the deposition of insoluble particles in the lung.

Macrophages in Bronchus-Associated Lymphoid Tissue. A third general category of macrophages that is anatomically associated with the conducting airways consists of macrophages that are present in the bronchus-associated lymphoid tissue (BALT) (*154,174,175*). Arguably, macrophages in this category could equally as well be arbitrarily classified as an anatomical subset of the lung's population of interstitial macrophages (IM). Both dendritic and interdigitating type macrophages have been reported to occur in BALT (*174,175*). Based on antigen expression and enzyme-cytochemical labeling (*154*), macrophages in rat BALT actually consist of three further subpopulations. The majority (~70%) of the BALT macrophages, which, like AM, AI-LM, and blood monocytes (*154*) positively label with the monoclonal antibody ED1, are scattered throughout the BALT. Another subpopulation of macrophages that border BALT positively labels with the monoclonal antibody ED2, which recognizes an antigen expressed by IM resident in perivascular and peribronchial connective tissue (*153,154*). The third subpopulation of BALT macrophages consists of cells that do not express the antigen recognized by either ED1 or ED2. In this regard, the ED1- and ED2-negative macrophages in BALT resemble IM in the alveolar septal region, which apparently are also negative for the antigens recognized by the ED1 and ED2 antibodies. Thus, the different antigenic profiles of the BALT macrophages in the rat, at least, suggest they could originate from AM, from AI-LM, from perivascular and peribronchial IM, from parenchymal IM, and even from blood monocytes that have translocated to the BALT regions, although other postulated explanations for the three different phenotypes of BALT macrophages are presently equally as tenable (*154*).

Quantitative information about the numeric contribution macrophages in BALT make to the total size of the pulmonary macrophage population in healthy lungs of species that contain such sites is unknown. In the rat's lung, at least, BALT macrophages would normally be expected to be relatively few in number compared to the numbers of AM and other interstitial macrophage subpopulations because there are only about 30–50 BALT sites in the rat's lung (*176*), and lymphocytes account for the vast majority of cells in BALT (*177*). The size and perhaps numbers of these sites, and the numbers of macrophages they contain, evidently can increase, however, in response to particle deposition in the lung (*48*).

The role BALT macrophages play in the clearance and retention of particles is unclear. While there is little doubt that some particles that deposit in the lung can be found in BALT macrophages at times thereafter (*48,178*) (Fig. 14) how the particles originally arrive to BALT sites requires further study. One possible mechanism is the passage of freshly deposited free particles across and/or between airway epithelial cells and the engulfment of the translocated particles by resident BALT macrophages. Several investigators have found evidence that airway epithelial cells can endocytize particles, and that some materials deposited in the airways can penetrate between airway epithelial cells (*91,179–181*). In addition to direct particle encounters with airway epithelial cells shortly after particle deposition in the airways, it is also conceivable that particle–epithelial cell encounters and the subsequent uptake of particles by airway epithelial cells may result from the release (*135*) or perhaps the direct conveyance (*133*) of particles by particle-laden macrophages that are in close apposition with the airway epithelial cells (*90,147*). Yet, some evidence argues against the transepithelial passage of particles as the means by which particles occur in BALT. As specific examples, Fournier and co-investigators (*182*) found no evidence for an incorporation of submicron-size latex microspheres into BALT after the particles were instilled into the tracheas of rats, and Bienenstock et al. (*183*) have reported that colloidal carbon did not appear in rabbit BALT after being instilled intrabronchially.

A second possible mechanism for the appearance of particle-laden macrophages in BALT is that they are particle-containing AI-LM that have migrated across the airway epithelium. This mechanism is conceptually similar to the suggestion by Lipscomb and co-workers (*184*) that macrophages exposed to particles in the luminal environment of the conducting airways may migrate into BALT. Some indirect support for this mechanism comes from Stirling and Patrick (*185*) who found particle-laden macrophages located subepithelially in the tracheas of rats 1 day after the animals were intratracheally instilled with submicron-sized, radiolabeled $BaSO_4$. This observation, in addition to electron microscopic observations that were interpreted to indicate macrophages can extend between tracheal epithelial cells, led these investigators to suggest that the appearance of particle-containing macrophages in subepithelial locations was due to the transepithelial migration of AI-LM.

A third postulated mechanism involves translocations of particle-containing AM and/or IM. Using trypan blue and carmin red to label rat lung macrophages to follow their migratory

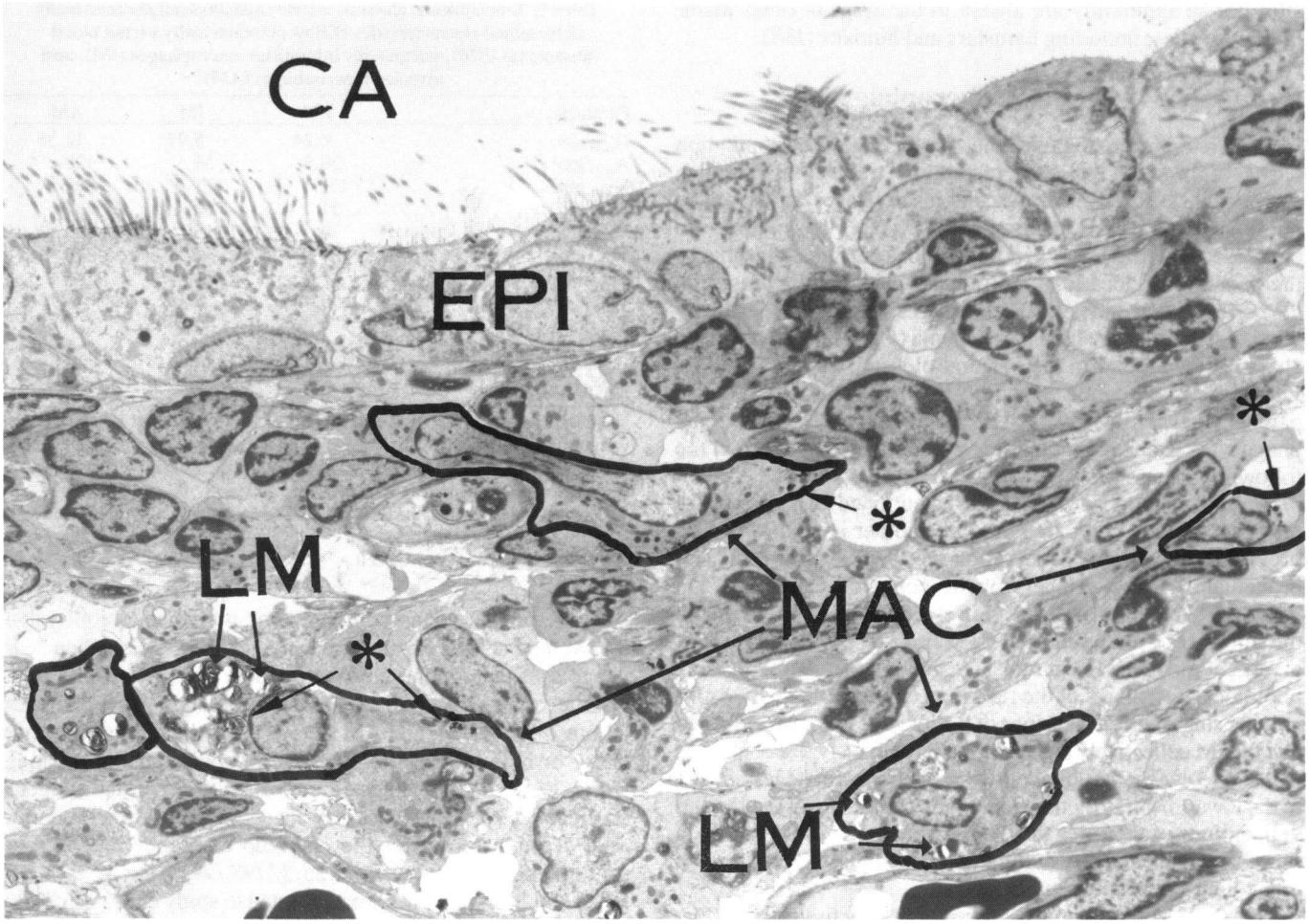

FIGURE 14. Particle (*/TiO_2)-containing macrophages (MAC) in bronchus-associated lymphoid tissue (BALT). (CA) lumen of conducting airway; (EPI) epithelial cells. The macrophages have been outlined for clarity. Like many alveolar macrophages, some of the BALT macrophages contain lamellar material (LM).

pathways in the lower respiratory tract, Brundelet (*157,186*) reported that most of the labeled AM penetrated into the interstitial connective tissue of the lung (where the AM by definition become IM) at numerous anatomical sites with one pathway being the passage of AM directly from alveoli to nearby peribronchial lymphoid tissue. As pointed out previously, however, conclusions drawn from this study are complicated by the fact that the animals used in the study were diseased. Nevertheless, we have observed that some of the macrophages in BALT that contain particles after particle depositon in the lung do resemble AM in morphologic appearance (Fig. 14).

A fourth potential source of particle-containing macrophages in BALT may be IM that have migrated or were otherwise transferred to BALT sites after they encountered and engulfed particles that passed epithelial barriers and passed into lung interstitial sites. As of this point in time, evidence for this possibility is at best suggestive (*154*). Still another possible explanation for the appearance of particles in BALT macrophages is that the particles arrived at the BALT sites as free particles after gaining entry into the lung's interstitial region, and these free particles are simply engulfed by the macrophages resident in the BALT.

However particles ultimately gain access to BALT, their fate thereafter continues to be problematic. Based mainly on the earlier reports of Brundelet (*186*), Tucker et al. (*158*), and Green (*99,187*), some investigators (*48,122*) continue to believe that particle-containing macrophages can exit BALT onto the surface of the conducting airways where they undergo removal from the respiratory tract by mucociliary transport, whereas the results of other studies have led other investigators to express doubt about the importance of this pathway for the clearance of particles (*90*). Even so, the results of more recent observations that dust-laden macrophages in lungs heavily burdened with TiO_2 pass from peribronchial lymphoid tissue into the airway lumen further suggests that such a pathway may exist under some circumstances (*48*). What other pathways particle-laden macrophages in BALT may ultimately follow remains to be determined. Decisive evidence that particle-containing BALT macrophages may leave BALT sites and translocate to other interstitial sites and perhaps lymphatic channels, either by ameboid migrating or by more passive transport with interstitial fluid flow, is currently nonexistent. Thus, it remains possible that insoluble particles in BALT may be retained there essentially indefinitely. Lastly, it should be pointed out that while macrophages in BALT may play a role in the clearance or retention of particles in the lower respiratory tracts of some species, e.g., rabbits, rats, mice BALT

sites per se apparently are absent in the lungs of other mammalian species, including hamsters and humans (*188*).

Pulmonary Interstitial Macrophages

Overview. Next to the AM, the second largest subpopulation of macrophages in the lower respiratory tracts of mammalian species that do not have intravascular macrophages consists of the pulmonary interstitial macrophages (IM), which, in turn, are composed of numerous subset populations that are defineable by their anatomical locations. Cells in this category are present in the peribronchial and perivascular spaces (*90,153,154*), in the interstitial spaces of the lung parenchyma (*9,33,189*) in lymphatic channels (*146*), and in the visceral pleural region (*190–193*). Langerhans and dendritic type cells also occur in the lung's parenchymal region (*169*), but because their role as professional phagocytes is questionable, they will not be considered in the following discussion.

How the macrophages in the aforementioned anatomical sites may functionally compare with one another is unknown. The previously mentioned observation that perivascular and peribronchial IM in the rat label with the monoclonal antibody ED2 while the IM in the alveolar region evidently do not suggest that the lung's IM population may be composed of phenotypically divergent members. Regardless, in good agreement with morphologic assessments of the rat's lung (*9*), *in vitro* analyses involving single cell suspensions of the lungs of rats have indicated that the IM collectively account for approximately 2% of all cells in the alveolar region, or about 40% of all macrophages present in the lower respiratory tract (*10*). Information about the IM and delineations of the roles these cells play in the lung have been limited by their inaccessibility due to their anatomical location in the lung's interstitial matrices.

Like the AM, the origin(s) of the IM in lung tissue needs further clarification. Some lines of evidence suggest that the IM may be derived from circulating blood monocytes (BM), and that they may represent an intermediate, transitional stage between BM and AM before they further translocate into the alveoli (*32–34*). Other investigators have suggested that while the lung's population of IM may contribute to the AM population, at least some of the IM may not originally be of BM origin (*194*). In a recent ultrastructural/morphometric study of flow cytometrically sorted AM, IM, and BM (*195*), we obtained evidence that is generally consistent with the postulate that the IM are an intermediate, maturational stage between the BM and the AM in that for virtually all structural end points analyzed, the IM were found to have cellular and ultrastructural features that were quantitatively between those of the BM and AM (Table 1). This comparative study also revealed that the IM are structurally much more akin to the BM than they are to the AM, and essentially no evidence was found that suggested the IM mature to the AM phenotype while in the interstitium. (These same findings, incidently, also suggest that AM normally do not significantly contribute to the lung's IM population.) If the IM do in fact contribute to the homeostatic maintenance of the size of the AM population under steady-state conditions, our findings suggest that their final transition into typical AM occurs after entry onto the alveolar surface. Even though the results from the above study are generally consistent with the possibility that the lung's IM population represents an intermediate maturational stage between putative BM as precursors and further differentiated or more activated AM, they are also consistent, however, with the possibility that the IM may, at least in part, be a resident lineage of mononuclear phagocytes that is generally independent of a BM origin (*196*). To date, other analyses of antigenic differences and similarities among the BM, IM, and AM have as yet to distinguish whether either or both of these possibilites are operational in the lung (*14,15,20,154,197,198*) in that the expression of surface antigens on mononuclear phagocytes may change as they further differentiate or mature or after they take up residency in different anatomical compartments (*13,23,27,198,199*).

Table 1. Transmission electron microscopically/morphometrically determined characteristics of flow cytometrically sorted blood monocytes (BM), pulmonary interstitial macrophages (IM), and alveolar macrophages (AM).[a]

Parameter	BM	IM	AM
D_{cell}, μm	6.24	6.97	12.36
A_{cell}, μm^2	30.6	38	120
V_{cell}, μm^3	127	177	989
V_{nuc}, μm^3	51	62	137
V_{cyt}, μm^3	76	115	853
V_{mit}, μm^3	7	9	40
V_{lys}, μm^3	3	21	144
Vv_{nuc}	0.405	0.352	0.138
Vv_{cyt}	0.595	0.648	0.862
Vv_{mit}	0.059	0.051	0.038
Vv_{lys}	0.028	0.017	0.136
No. lysosomes/cell[b]	410	912	5323

Abbreviations: D_{cell}, mean cell diameter; A_{cell}, mean cell area; V, mean volume of a given feature in an average cell, i.e., whole cells (cell), nuclei (nuc), cytoplasm (cyt), mitochondria (mit), lysosomes (lys); Vv: volume fraction each cellular feature contributes to the average whole cell.

[a]The methods by which the BM, IM, and AM were isolated for this study are described elsewhere (*204*).

[b]Average number per mean cell.

Several investigators have attempted to study cells nominally referred to as IM or implied to be IM using only mechanical disruption of lung tissue to liberate study cell populations (e.g., *200–203*). As we have recently shown, however, the results from these investigations are questionable because the tissue disruption techniques used mainly procure unlavaged AM and not IM (*204*). In other studies, investigators have attempted to characterize the functional characteristics of the IM contained in cell populations harvested from enzymatically dissociated lung tissue. Findings obtained in these *in vitro* investigations are also difficult to interpret because precautions were not taken to eliminate AM and other contaminating cell types from the experimental samples. Nevertheless, some of these latter studies have suggested that the IM do differ from AM and/or BM in a variety of respects that may be relevant to their roles in lung clearance, including their expression of Fcγ and C3 receptors (*205,206*) and their abilities to phagocytize latex particles and zymosan (*207*). More recently, various characteristics of IM have been more directly investigated by exploiting antigenic differences between the IM and AM in order to flow cytometrically sort viable IM from enzymatically dispersed lung cell suspensions (*15*), or after isolating IM from single-cell suspensions prepared from whole lungs using an Fcγ receptor affinity technique in conjunciton with flow cytometry (*208*). These studies have revealed that IM indeed are capable of performing Fcγ receptor-mediated phagocytosis, and that IM can phagocytize zymosan (*15,208*). It should be noted, however, that IM isolated from single cell suspensions of lung tissue may not necessarily

represent findings that would be obtained if technical approaches allowed the separate procurement of each anatomically defined subpopulation of IM. Until further morphometric analyses provide additional quantitative information about the relative numbers of IM that are present in the various interstitial compartments, and until each IM subpopulation can be isolated alone and unique identifying characteristics (if existent) are assignable to the IM subpopulations, the relative contribution each anatomically defined IM subpopulation makes to the lung's total IM population in lung cell suspensions remains speculative at best.

Roles in Particle Clearance. Information about the roles the IM play in the clearance and retention of particles has been derived mainly from electron microscopic studies of lung tissue performed at various sequential times after particle deposition. Several of these studies have demonstrated that some particles in the alveoli can escape from being phagocytized by Am, and that these particles can be endocytized by type I pneumocytes as an early first step by which particles can be subsequently transported into the alveolar interstitium (*77,81*). An outcome of the endocytosis of particles by the type I epithelial cells is the transepithelial vesicular transport and release of free particles into the interstitium (*38,81*), where they can remain as free particles for some undetermined period of time and/or be engulfed by the IM (Fig. 15). The relative abundance of particles that are taken up by the type I cells appears to be a function of the numbers (and probably sizes) of particles deposited, with greater uptake occurring as acutely deposited lung burden loads are increased (*81*). Such findings led Adamson and Bowden (*81*) to postulate that when high lung burdens of particles exceed the capacity of resident and recruited phagocytes to engulf the particles, the presence of free particles on the alveolar surface increases the likelihood of particle encounters with type I cells and their subsequent interstitialization. A hypothetical extension of this postulate is that some fraction of deposited particles gain entry into type I pneumocytes even when lung burdens are low and the phagocytic mechanism is not overwhelmed. Otherwise, the process of phagocytosis, i.e., particle encounters and engulfment by AM, seemingly would have to be accomplished with complete efficiency within a post-depositional time span that would preclude particle uptake by the type I cells. The above extension of the Adamson and Bowden postulate is consistent with numerous reports that particles appear in the regional lymph nodes even when they are deposited at relatively low lung burdens (*105,106,118,132*). Assuming particle phagocytosis by AM is not perfectly efficient and sufficiently rapid, increasing numbers of particles would be expected to gain entry into type I cells essentially continuously during chronic aerosol exposures even if the phagocytic capacities of alveolar phagocytes have not been ex-

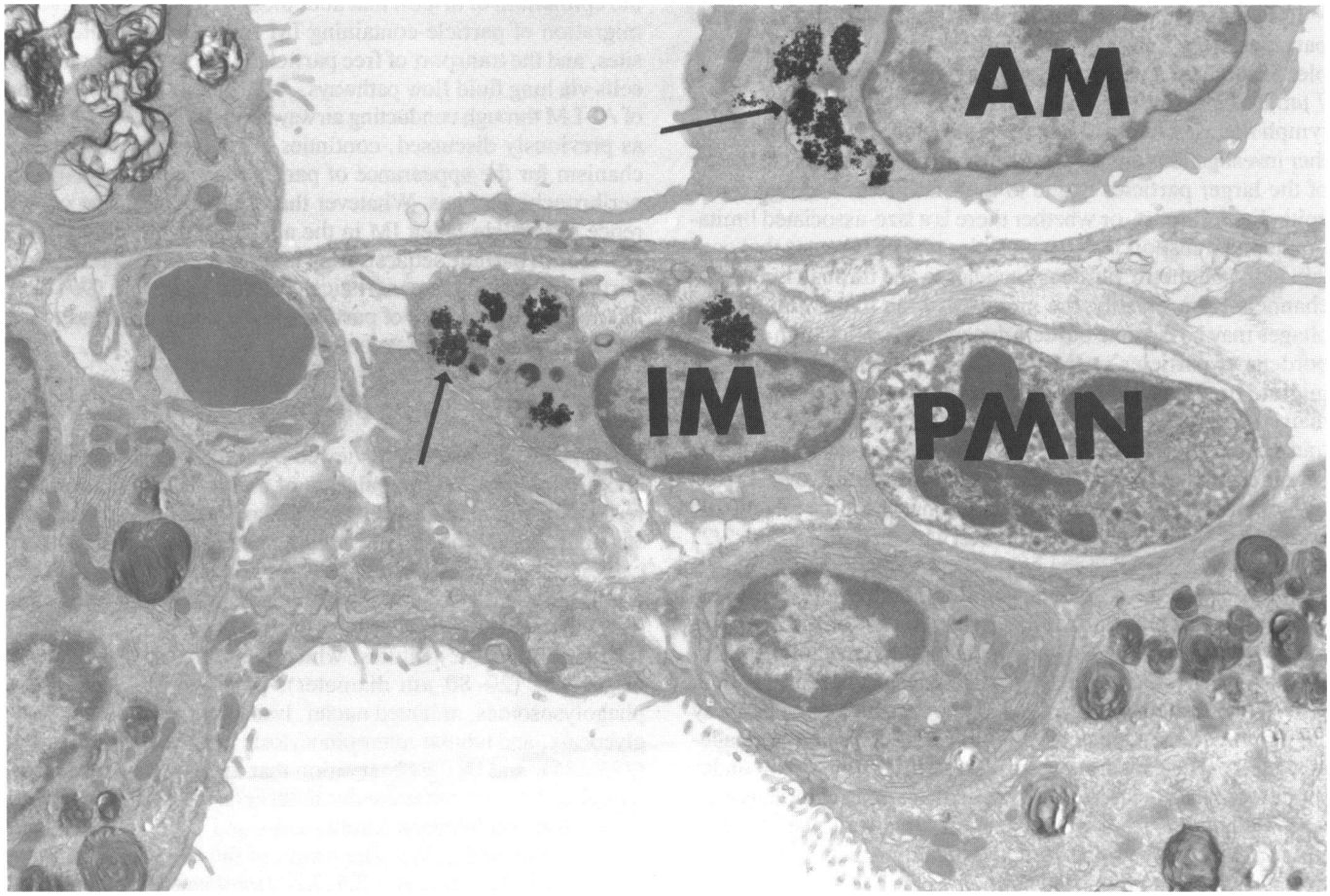

FIGURE 15. Particles in an interstitial macrophage (IM) following aerosol exposure to TiO_2 delivered over a 90-day period. In addition to containing TiO_2, the IM has also engulfed a polymorphonuclear leukocyte (PMN).

ceeded. Additionally, as particles are gradually released on a continual basis by alveolar phagocytes that initially engulfed them (*64,130,132*), a fraction of these free particles may also undergo passage from the alveolar surface into the type I cells at times well after initial particle deposition.

The fate(s) of the interstitialized free particles and IM that phagocytize them continues to be a subject in need of further research. Some particles that gain entry into the interstitial compartment can further translocate to the extrapulmonary regional lymph nodes after entering lymphatic capillaries as free particles or as particles contained in IM (*4,48,116,118,120*). Free particles have been shown to penetrate the lymphatic channels through clefts between the endothelial cells that form the lymphatic pathways. They also can enter the lymphatic channels after they are endocytized by the lymphatic endothelial cells (*113,209*). Whether particle-containing IM gain access to the lymphatic channels via passive transport by lymphatic fluid flows, or whether an active migration of IM is involved is not known. Once inside the lymphatic channels, however, their further transport to draining lymph nodes probably is mainly passive. What fraction of particle-containing IM may ultimately be translocated through lymphatic channels to regional lymph nodes and the factors that determine whether a particle-laden IM will enter this lung clearance pathway instead of remaining locally stationary or following some other translocating pathway are unknown.

It is known, however, that the overall process of the passage of particles to the lymph nodes is particle-size limited. For example, Snipes and co-workers (*105,106*) found that particles up to 7 μm in diameter translocated from the lungs of animals to the lymph nodes, whereas particles larger than 9 μm did not. Further investigations are required to determine if the lack of transfer of the larger particles is due to their failure to undergo transepithelial transport, or whether there is a size-associated limitation in the transport of large particles and phagocytes that contain high volumetric burdens particles to and through lymphatic channels. Conceivably, the mobility of the interstitial macrophages may be particle burden-dependent, so that high cellular burdens of particles in IM may compromise their abilities to migrate or otherwise traverse through the complex interstitial matrix. Whatever the mechanism(s), it is evident that the mass transport of particles from the alveoli to the regional lymph nodes becomes greater as lung burdens increase, with greatest mass tranlocations occurring under conditions where the phagocytic capacity of alveolar phagocytes is overwhelmed (*81,118,210–212*) and more free particles can gain access to the interstitium.

In addition to the lymphatic pathway, some evidence also indicates that interstitialized particles can leave the lung via transport of particle-laden macrophages into the pulmonary circulation when lung burdens of particles are extraordinarily high (*48*). The mechanisms involved in this lung clearance pathway are not understood, and compelling evidence for macrophage-mediated particle clearance to the pulmonary circulation under conditions where acquired lung burdens are more commensurate with realistic environmental exposures has yet to be obtained (*100*). Conceivably, some particle-containing macrophages and free particles observed in the pulmonary vasculature under conditions of high lung burdens could represent constituents emitted in the efferent lymph from lung-associated lymph nodes where the retention of particle-engorged macrophages may be incomplete.

Some particle-laden IM apparently can also migrate across the alveolar epithelium and become members of the AM population (*77*). Evidence for this phenomenon has been mainly observed shortly after particle deposition in the lung. What fraction of the interstitial macrophages may subsequently migrate over time into the alveoli with their engulfed burdens and thereby contribute to the resident AM population during alveolar clearance remans a problematic issue in need of future research. Moreover, no investigations have been conducted to date to assess the influence that an IM's burden of particles may have on its ability to enter the alveolar space compartment from the interstitium, assuming that the IM do indeed follow this pathway. How particles that gain entry into the interstitium may affect the size of the IM population and the interstitial recruitment of other phagocytic cells separately from events associated with particles in the alveolar space compartment per se, are current problem areas in need of exploration.

Another source of current uncertainty concerning the IM comes from observations that retained lung burdens over time can increasingly accumulate in the IM located in perivascular (both arteriole and venule), peribronchiolar, and visceral pleural connective tissue sites (*38,48,77,90,213,214*) (Figs. 16–18). Possible explanations for the appearance of particle-laden IM at these sites have included the passage of free particles and/or AM across the epithelium of alveoli that abut these structures, the gradual migration of particle-containing IM from alveolar interstitial sites, and the transport of free particles and particle-containing cells via lung fluid flow pathways. Additionally, the penetration of AI-LM through conducting airway epithelium (*90,91,158,215*), as previously discussed, continues to remain a potential mechanism for the appearance of particle-laden macrophages at peribronchiolar sites. Whatever the mechanism(s), the occurrence of particle-laden IM in the above sites may collectively represent a particle sequestration compartment with long-term particle retaining characteristics. Sorokin and Brain (*90*) have likened the appearance of particles in the connective tissue IM to a kind of pulmonary tattoo.

Intravascular Macrophages

Overview. As recently reviewed by Warner and Brain (*216*), another bona fide subpopulation of macrophages is normally resident in high abundance in the capillary beds of the pulmonary vasculatures of some mammalian species, including sheep, calves, pigs, goats, cats, and perhaps horses (*216–226*). These pulmonary intravascular macrophages (PIM) can be distinguished from blood monocytes because of their more differentiated morphologic features, which include a relatively much larger size (20–80 μm diameter), prominent phagosomes, phagolysosomes, indented nuclei, lysosomal granules, a fuzzy glycocalx, and tubular micropinocytosis vermiformis structures (*223,225*), and by the observation that the PIM form adhesive complexes with capillary endothelial cells (*223,225*) similar to those observed between Kupffer cells and endothelial cells in hepatic sinusoids (*228*). The origin of the PIM remains to be determined. Warner et al. (*225*) has speculated that they may be derived from bone marrow cells that attach to pulmonary endothelial cells or bone marrow-derived cells that enter the

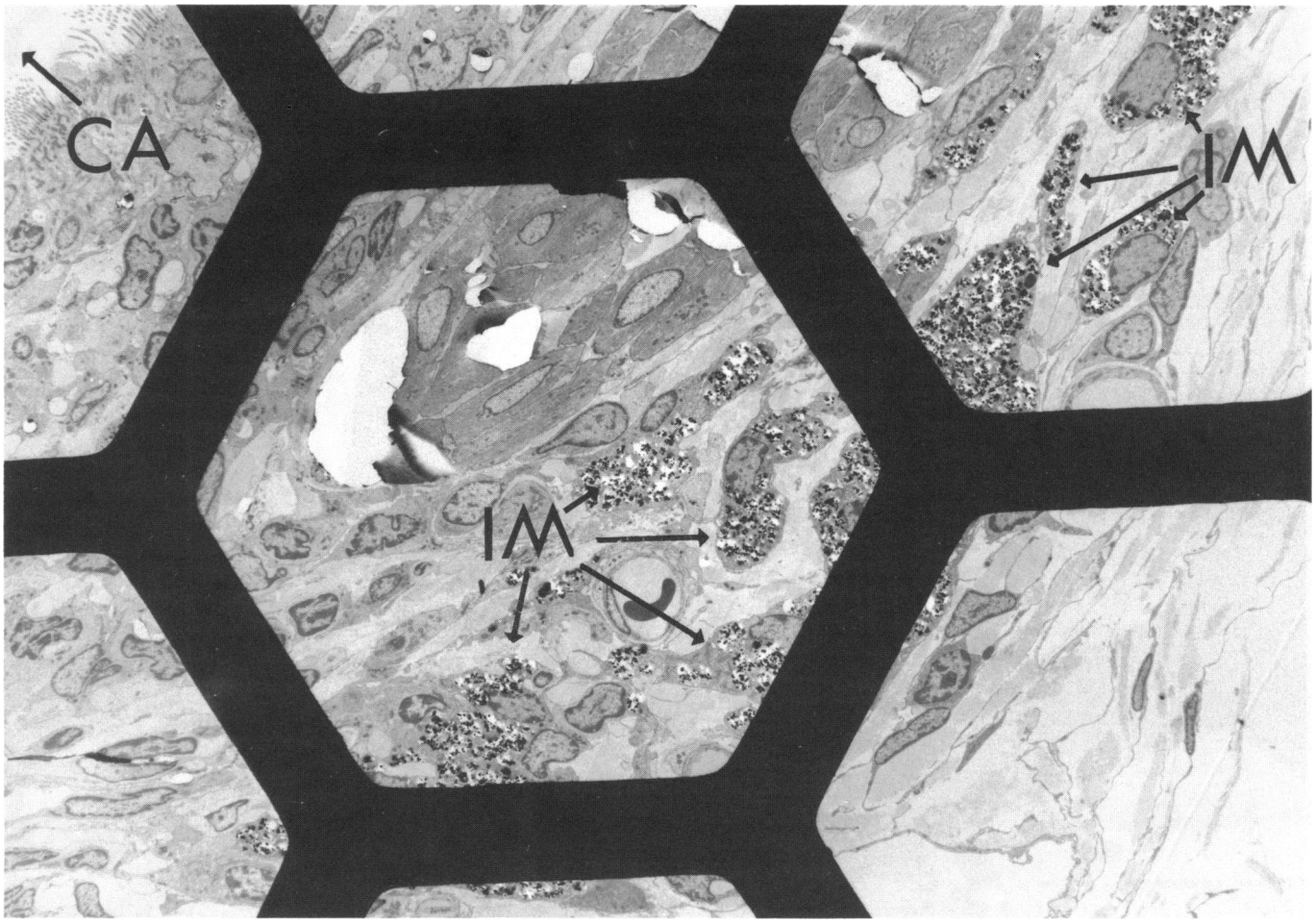

FIGURE 16. Particle-laden peribronchiolar interstitial macrophages (IM) after a rat was exposed to aerosolized TiO_2 that was delivered over a 90-day period. (CA) luminal surface of a conducting airway.

pulmonary interstitium and subsequently migrate into the capillary lumen. These same investigators also suggested that the PIM may be a self-replicating population of lung macrophages. Firm information is not currently available about the relative fractional contribution PIM make to the total numbers of all pulmonary macrophages in the lungs of the above species. However, Werner and co-investigators (*225*) have estimated that there may be almost three times as many PIM in the lungs of sheep than there are AM. Recent observations suggest that PIM may also be present in the pulmonary capillaries of humans (*229*), but some experts in the area of PIM biology believe that very few exist in the human lung under normal circumstances (*216*). Few, if any, PIM are resident in the pulmonary vasculature of other mammalian species that are often used studies involving the deposition of particles in the lung, including rats, rabbits, hamsters, guinea pigs, and dogs (*220,227*). Whether the presence of PIM can be induced under some conditions in the lungs of species that do not usually contain them has not been firmly determined, although some recent investigations suggest that this may be possible (*230,231*).

Particles and PIM. A main defensive function of the PIM is the removal of particles introduced into the pulmonary vasculature via the venous circulation, the removal of effete erythrocytes, and the engulfment of PMN and cellular debris during inflammation (*216,224,226,232,233*). The ultimate fate of the PIM once they contain particles requires further investigation. Some evidence does suggest, however, that their continued presence in the lung's vasculature may be prolonged (*234*). What role the PIM may play in the removal of particles that deposit in the alveoli is also unclear. If particles that deposit in the alveoli can make their way through epithelial and endothelial barriers and emerge in the pulmonary vascular bed, it would seem likely that they would be subject to being phagocytized by the PIM. Additionally, if free particles enter the venous circulation via lymph fluid flowing from regional tracheobronchial lymph nodes, it seems reasonable to postulate that such particles may be engulfed by the PIM as well.

Lung-Associated Thoracic Macrophages

Pleural Macrophages

Overview. The pleural space, which is considered by some investigators to be an interstitial space compartment (*235*), occurs between the visceral pleura that covers the surface of the lung

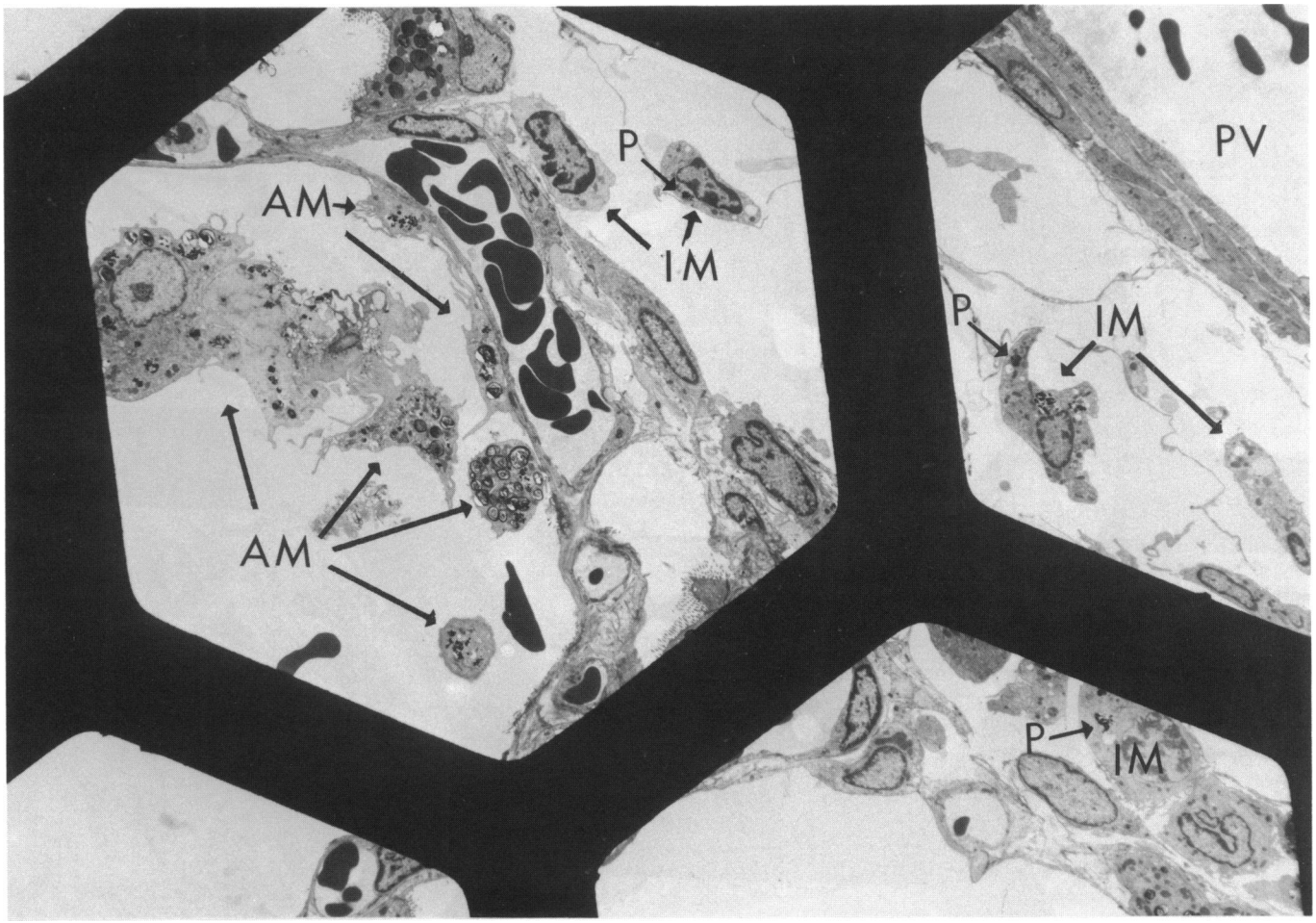

FIGURE 17. Particle-containing interstitial macrophages (IM) located in the perivascular region following aerosol exposure to TiO_2 delivered over a 90-day period. (AM) alveolar macrophages; (PV) pulmonary venule; (P) TiO_2 particles.

and the parietal pleura that covers the mediastinum, diaphragm, and inner surface of the thoracic cage. In the human, the pleural space contains a small volume of fluid that fills an estimated 20-μm wide gap between the visceral and parietal pleura. A variety of cell types are contained in the pleural space, including macrophages. Like some of the other previously discussed macrophage populations, the pleural macrophages (PM) have as yet to be well studied, even though they can be rather easily obtained with other pleural free cells by lavaging the pleural cavity, and they can be viably isolated in high states of purity (*87,236–238*). Until further information is obtained about the PM, it can only be assumed that they play important defensive roles in maintaining a healthy status of the visceral and parietal pleurae, and that they may be involved in the development of some lung disease processes (*239*).

In the human, PM account for about half of all cells present in the pleural fluid, with the remaining balance of cells being lymphocytes, granulocytes, mesothelial cells, and degenerated cells (*240*). The actual numbers of PM present in the human is unknown; PM have only been counted in pleural fluids recovered from normal subjects and not by extensive washings of the pleural cavity. In the mouse (BALB/c), approximately 3×10^6 cells can be washed from the pleural cavity and approximately 50% of the cells recovered from this species are PM (*236*). In the rabbit, cells with morphologic features of blood monocytes and macrophages collectively account for approximately 18% of the cells present in pleural fluid (*241*), but quantitative information on the total number of these cells present in the pleural space of the rabbit, as is best determined by lavage of the pleural cavity, has not been reported. In the normal adult Fischer-344 rat, approximately 1×10^7 pleural cells can be harvested by lavaging the pleural cavity, and approximately 20–50% of the recovered cells are PM with the other cell types being mainly mast cells, eosinophils, and lymphocytes (*62,237*) (Fig. 19). Although it seems evident that at least some of the PM are normally suspended in pleural fluid, and thereby essentially continuously mobile under the influence of ventilatory excursions, the possibility has not been ruled out that others may reside in close apposition with the mesothelial lining and are less mobile.

In vitro analyses of mouse PM have demonstrated that the PM have electronic volumes that are smaller than those of AM but that are similar to thosse of peritoneal macrophages (*236*). In a variety of other histoenzymatic and functional respects, the PM and peritoneal macrophages are more like one another than they

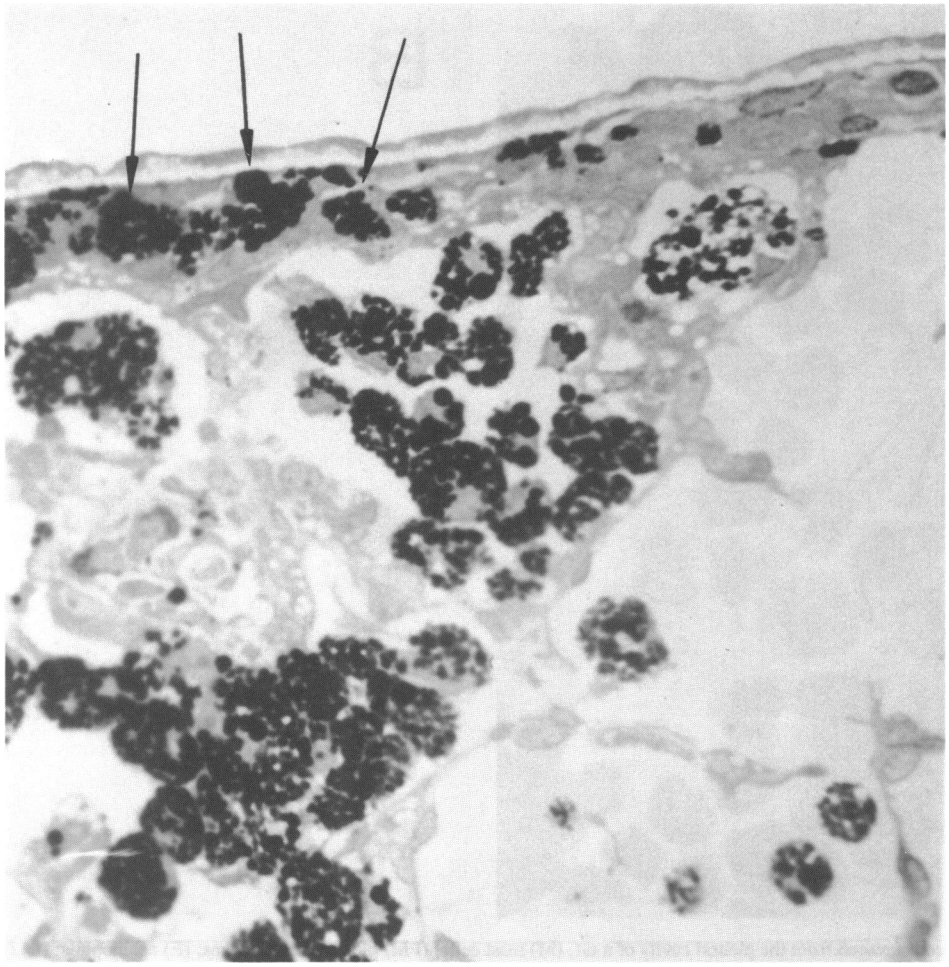

FIGURE 18. Light micrograph of a mouse lung on day 5 after the intratracheal instillation of carbon particles. Some of the particles are contained in macrophages (arrows) in the visceral pleural region.

are to AM (*236*). In the rat, the PM are also smaller than the AM, and they show less nonspecific esterase and Fcγ-receptor-mediated phagocytic activities than do AM (unpublished observations). The majority of both rat PM and peritoneal macrophages also possess a membrane antigen recognized by the monoclonal antibody 1G5 that is usually absent on the surfaces of AM (*242*). Some limited evidence has suggested that the PM originate from peripheral blood monocytes that migrate across the mesothelial lining of the pleurae (*239,243,244*), but it is also possible that self-replication of the PM in the pleural space may be involved in maintaining the size of the PM population under homeostatic conditions.

Responses to Particle Deposition in the Lung. The PM population apparently can be affected by the deposition of some types of particles in the lung. For example, Oberdorster and coworkers (*87*) reported that the deposition of particulate amosite into the rat's lung results in both an acute and a sustained recruitment of blood mononuclear phagocytes into the pleural space compartment, as indexed by increases in the presence of peroxidase positive macrophages. Unlike the lung's free-cell response to the amosite, the response in the pleural space did not have a PMN component. Moreover, no fibers were detected in the pleural lavage fluids during the cellular response in the pleural space.

In another study, we also investigated how the cell numbers and types in the pleural space (*62*) might alter during the lung's free cell response to the intrapulmonary deposition of a relatively high burden of less cytotoxic polystyrene microspheres. No acute influx of PMN into the pleural space accompanied the early recruitment of PMN in the alveoli in response to the particles. Similar to the AM response, however, PM numbers were also increased 1 day after particle deposition in the lung, and their numbers remained increased for at least 13 days thereafter. Based on observations made in another study (*128*), the increase in PM numbers observed in our study was not related to a passage of free particles or particle-containing AM into the pleural space compartment. Also in our study, the increases in PM numbers were not associated with any detectable increases in the occurrence of myeloperoxidase-positive macrophages. Conceivably, the increases in PM numbers observed in our study and that of Oberdorster et al. (*87*) could have been due to mononuclear phagocyte-specific chemotactic and mitogenic factors that gained entrance into the pleural space from the alveoli following free-cell–particle interactions (*74*). On the other hand, the results

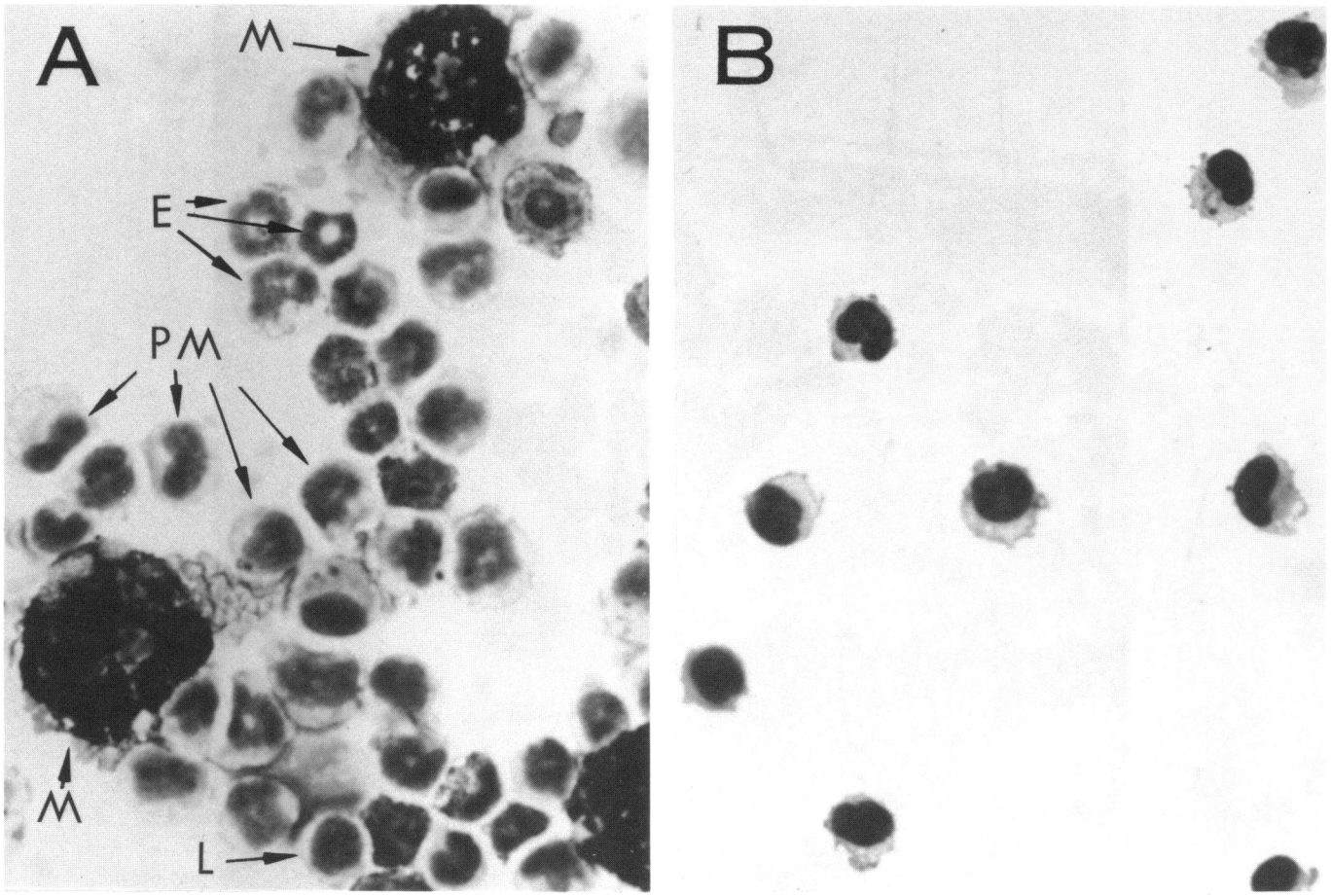

FIGURE 19. (*A*) Pleural cells lavaged from the pleural cavity of a rat. (M) mast cells; (PM) pleural macrophages; (E) eosinophils; (L) lymphocytes. (*B*) Pleural macrophages that were flow cytometrically sorted from a pleural free-cell population.

obtained in both of the above studies show that chemotactic factor(s) that must be involved in the recruitment of PMN into the alveoli in response to particle deposition do not necessarily reach the pleural space in biologically significant or persistent concentrations, or alternatively, they are otherwise inoperable in the pleural space compartment. Regardless, the above investigations have demonstrated that particle deposition in the lung can affect PM in the pleural space, and they underscore the need for further studies of the changes that can occur in the pleural free-cell population following the deposition of environmental agents in the lung and for investigations of the mechanisms that underlie such changes.

Responses to Particles in the Pleural Space. As indicated previously, some types of inhaled particles, especially fibers, can gain access to the pleural space within days after deposition in the lung. Once particles are in the pleural space, they are presumably susceptible to being phagocytized by PM. As well, PM probably play a routine janitorial role by phagocytizing degenerated, exfoliated mesothelial cells and other biotic materials that may enter the pleural space, with this function likely being most pronounced during active pleural effusion.

Similar to the alveolar compartment, a free-cell response also occurs in the pleural space upon the direct introduction of particles or after the intrapleural instillation of a wide range of nonparticulate materials, including only saline and phosphate-buffered saline (*238,239,245–249*). Thus, information collected to date suggests that the acute deposition of virtually any material into the pleural space, however innocuous, results in the recruitment of at least some inflammatory cells. Studies that have specifically focused in detail on changes in the pleural free-cell population in response to particle deposition are few in number. Following the instillation of a high burden of chrysotile asbestos (150 mg) into the pleural spaces of rabbits, for example, Sahn and Anthony (*239*) found a marked increase in PMN 24 hr later. PM numbers increased at 48 hr, subsequently peaked at 72 hr, and remained elevated for at least 120 hr after the instillations. At later times, almost all of the pleural free cells were lymphocytes. In other rabbits that were rendered neutropenic with nitrogen mustard prior to the asbestos instillations, the PMN response was minimal, and the PM population did not increase in size. In that nitrogen mustard does not appear to impair circulating monocytes (*250*), and because the PM response could be restored when PMN were replenished into the pleural spaces of nitrogen mustard-treated animals, Sahn and Anthony (*239*) postulated that PMN in the pleural space release a chemotaxin that in turn serves to recruit mononuclear phagocytes.

In another investigation, Antony and co-workers (*244*) also found similar increases in pleural PMN and PM after Bacillus

Calmette-Guerin was intrapleurally administered to rabbits. Using more benign polystyrene microspheres (4×10^8, 2 μm diameter) and an intrapleural instillation technique that precludes co-administering the particles to the lungs, Lehnert and co-investigators (*238,251*) also investigated the free-cell response in the rat's pleural space. Similar to the aforementioned rabbit studies, the microspheres also brought about an early recruitment of PMN and an enlargement in the size of the PM population that persisted for about 1 month following particle deposition. Like the biphasic AM response to particle deposition in the alveoli, evidence was also obtained in this study that the PM response in the pleural space is also biphasic. Of significance, this study also uniquivocally demonstrated that PM are capable of phagocytizing particles that enter the pleural space, and, in addition to engulfing free particles, it revealed that one mechanism by which PM can obtain cellular burdens of particles is by the engulfment of particle-containing PMN. Moreover, evidence obtained in the study suggested that the chemotactic and perhaps mitogenic factors involved in the pleural free-cell response do not gain entry into the alveolar space compartment in that no qualitative or quantitative changes in the lung's free-cell population were observed as the pleural free-cell population was undergoing major alterations in response to the particles, and no evidence was found to suggest that particle-containing PMN and PM can migrate across the visceral pleura into the alveolar space compartment.

PM-Associated Removal of Particles from the Pleural Space. Given that PM can phagocytize particles in the pleural space compartment, the question arises as to what the fates of these cells are thereafter in terms of their clearance pathways from the peural space. Some information obtained mainly from earlier investigations has suggested that particle-containing phagocytes and other materials are removed from the pelural space via parietal pleural stomata and associated lymphatic channels (*193,252-259*), but this route of elimination may not be the only or most important pathway. More recently, Lehnert and colleagues (*238*) examined this matter as a component of the previously discussed study in which they investigated the pleural free-cell response to intrapleurally instilled microspheres. They found that approximately 80% of the instilled particles were removed (or at least were not recoverable by pleural lavage) from the pleural space compartment with an apparent half-time of about 0.3 days (Fig. 20) and that about 75% of the instilled particles were associated with the retrosternal caudal mediastinal tissue as of one day after the particles were administered (Fig. 21). A slower component of particle removal from the pleural space, which accounted for the remaining balance of particles and was mainly PM associated, was found to also have a relatively short half-life of approximately 6 days (Fig. 20). Histologic observations suggested that most of the particles associated with the caudal mediastinal tissue site were in mononuclear cells and PMN and that some of these cells were located in Kampmeier's foci (*260*) and in lymphatic channels. Closer inspection of this process by electron microscopy indicated that the association of the phagocytes with the retrosternal, caudal mediastinal tissue did not exclusively occur by cells simply passing into the tissue via stomata (*238,261*), although such a mechanism may have been an operable component as part of the overall process. These results, accordingly, suggest that the retrosternal, caudal

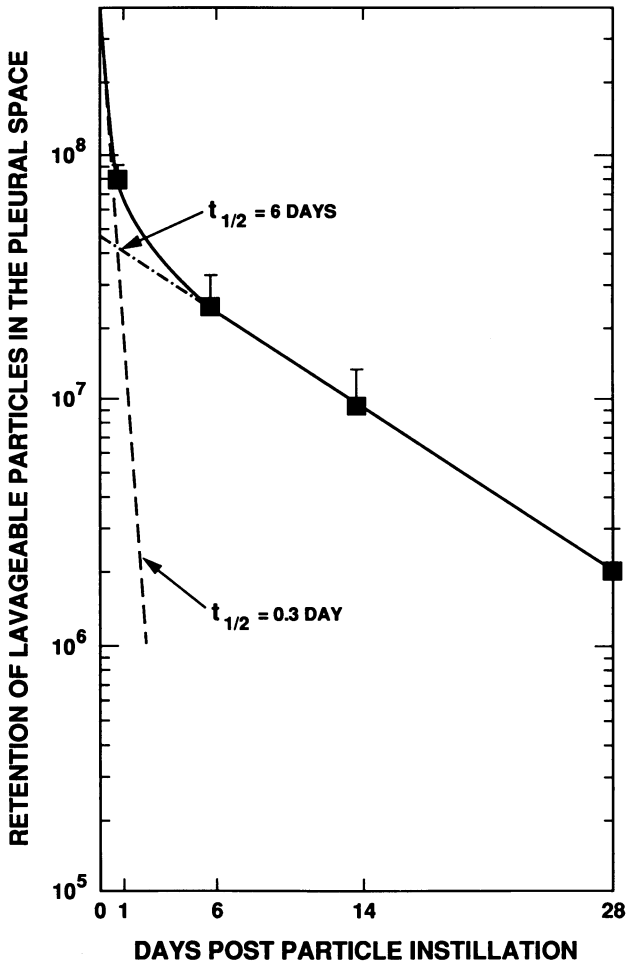

FIGURE 20. Retention characteristics of polystyrene microspheres.

mediastinal tissue is the primary route by which particle-containing PMN and PM are removed from the pleural space compartment. Furthermore, the rapid rate by which particles and particle-containing phagocytes appear to be removed from the pleural space via this pathway may underlie the relative lack of appearance of fibers in the pleural space usually noted in human subject whose lungs appear to contain appreciable burdens of asbestos in close proximity to to the visceral pleura. The ultimate fate of particles and particle-containing phagocytes that become associated with the caudal mediastinal tissue has as yet to be well investigated. However, lymph flow from the mediastinal pleura is thought to mainly drain to lymph nodes in the upper mediastinum and to tracheobronchial lymph nodes (*255*), which suggests the possibility that free particles and perhaps particle-containing pleural phagocytes may be translocated to these sites from the retrosternal, caudal mediastinal tissue.

Tracheobronchial Lymph Node Macrophages

Macrophages are normally present in relatively low abundance compared to the numbers of lymphocytes that are resident in the tracheobronchial or hilar lymph nodes that receive lymphatic drainage from the lung. Detailed information about subset populations of macrophages that may be present in these nodes is limited, although as many as three general subpopulations of

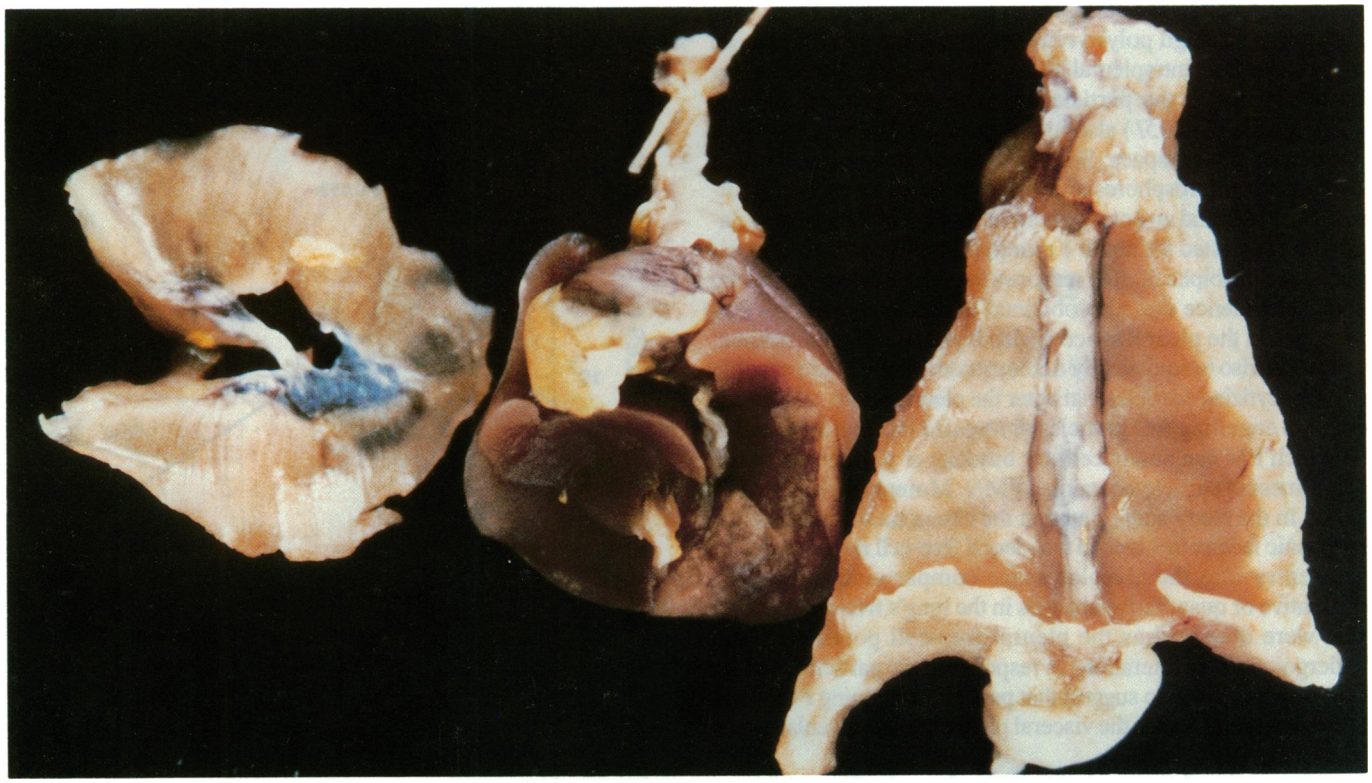

FIGURE 21. The majority of the microspheres (yellow-green) that were introduced into the pleural space of the rat became associated with the retrostenal, caudal mediastinal tissue located at the base of the heart. The mediastinal tissue is yellow-green in appearance due to the presence of microspheres.

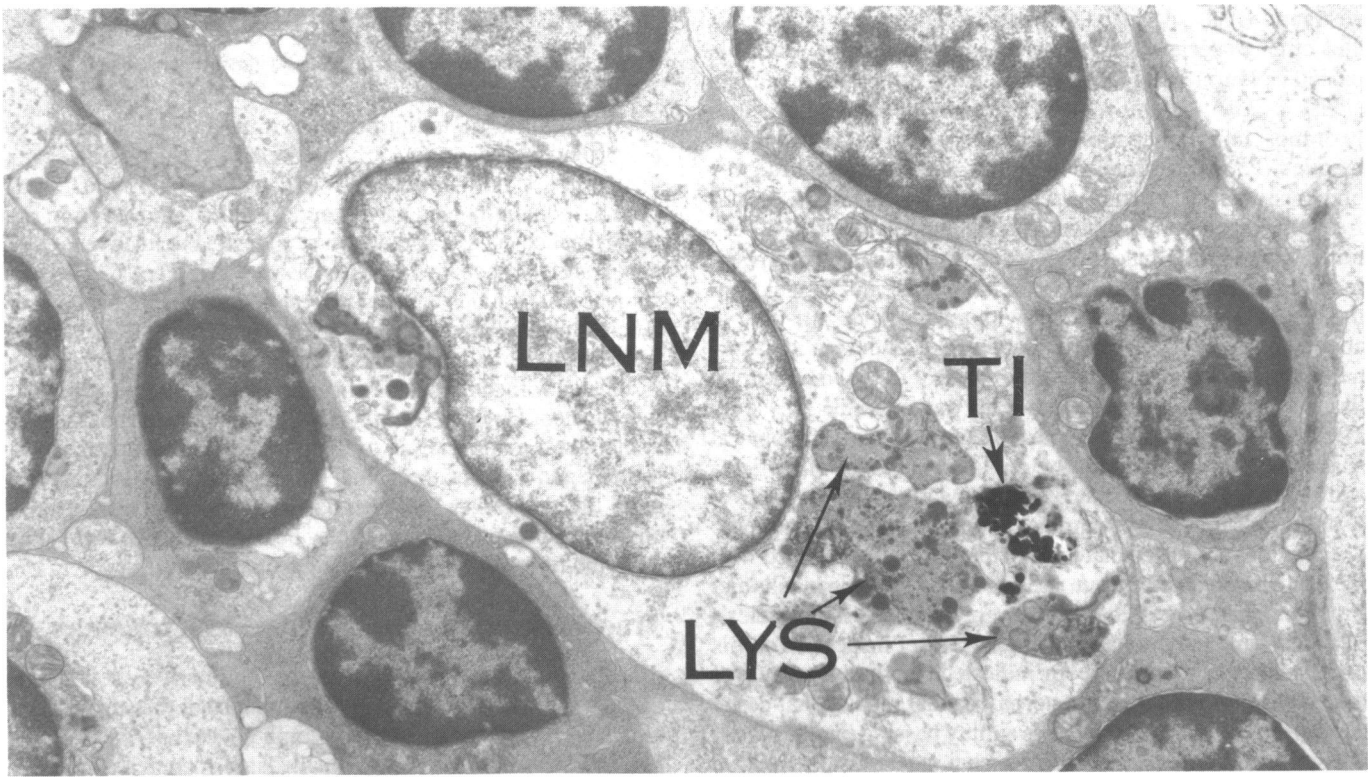

FIGURE 22. Particle-laden lymph nodal macrophage (LNM) in a tracheobronchial lymph node of a rat following exposure to TiO_2 (Degussa) over a 90-day period. (LYS) lysosomes; (TI) engulfed TiO_2.

macrophages have been identified in the hilar lymph nodes in the rabbit by their differeing morphologic features (*262*). Based on findings obtained with lymph nodes from other body sites, it appears likely that most of the macrophages in the lymph nodes are usually directly derived from immigrating blood monocytes (*262*). As pointed out previously, however, it seems evident that IM and perhaps AM may also contribute to the pool of macrophages in the tracheobronchial lymph nodes, especially after particle deposition in the lung (*48,113,264*) (Fig. 22).

The retention of particle-containing macrophages in the tracheobronchial lymph nodes has not been extensively investigated. Although the cells contained in efferent lymph are predominantly lymphocytes, small numbers of macrophages can occasionally be found as well (*265,266*). Such observations suggest the possibility free particles may exit the hilar lymph nodes in efferent lymph fluid and enter the blood for transport elsewhere. An expected outcome of this process would be the redistribution of particles to other body sites (*267*). The appearance of particle-containing macrophage as well as free particles in various body organs lends support to this possibility (*48,268-270*).

Summary

Numerous subpopulations of macrophages are known to exist in various anatomical subcompartments of the lower respiratory tract and in thoracic compartments that are associated with the lung. Each of these macrophage populations may in turn be composed of further subpopulations as defined by other morphologic, biophysical, functional, and biochemical criteria. General concepts about the roles the various subpopulations of pulmonary and thoracic macrophage perform in response to particle deposition in the lung and in the pulmonary clearance and retention of particles have developed over time. However, major gaps in our current understanding of these roles and their underlying mechanistic bases continue to persist in a variety of fundamental areas, including the origins of the macrophage subpopulations and homeostatic and particle-associated factors that govern their relative sizes and phenotypic expressions, the functional interrelationships among the macrophage subpopulations, macrophage translocation pathways, and, of utmost importance, how particle–macrophage interactions can lead to the development and progression of the many pulmonary diseased states that result from the deposition of particles in the lung.

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